PAN AFRICAN SANCTUARY ALLIANCE

PRIMATE VETERINARY HEALTH MANUAL

Second Edition / November 2009

PASA is committed to the conservation and care of African primates through the unique alliance of African sanctuaries.

PAN AFRICAN SANCTUARY ALLIANCE

VETERINARY HEALTHCARE

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INTRODUCTION TO THE FIRST EDITION

This is the first edition of the PASA Primate Healthcare Manual, which has been produced with the help of many participants at PASA conferences in Uganda, Cameroon and Kenya.

The aim is for all staff who work in one of the PASA sanctuaries to have access to useful and up-to-date information on many aspects of the veterinary management of primates in sanctauries. There is a huge range of knowledge and education in each of the staffs and, as such, it is difficult to know where to pitch the level correctly. We hope all people will gain something.

Our thanks go to the following participants and others that helped, including John Lewis, Jonathan Sleeman, Tom de Maar, Richard Ssuna, Peter Appel, Philipe Sarazin, Sherri Speede, Diccon Westworth, Christelle Colin, Nick de Souza, Crispin Mahamba, Peter Gray, Peter Jenkins, Ajao Adebowale and Egbetade Adeniyi. Particular thanks also go to our sponsors, including the International Fund for Animal Welfare, Great Ape Project, Zoological Society of London, Chester Zoo, Stichting AAP, Association of Primate Veterinarians, International Primatological Society, Wildlife Center of Virginia, Cincinnati Zoological Gardens, and Gorilla Haven. Thanks also go to Claire Cunningham for helping format the document and to ZSL for supporting this project.

Wayne Boardman, Eric Dubuis, Joanne Fielder and Steve Unwin
March 2004

INTRDUCTION TO THE SECOND EDITION

So here we are five years on with a second edition. This document is over twice the length of the first edition, and we hope people find this even more useful. There are many new chapters, including a current therapy section from previous workshop presentations which is constantly being added to, and virtually every other chapter has been expanded and brought up to date. This is still a field manual however, and not a textbook and as such will be updated as regularly as possible via the PASA website. Most of the references for this manual are also stored as PDF's on the website for further reference. We hope the manual fulfils our goal of helping veterinarians and their staff working in the sanctuary environment specifically, but also those vets working with primates in Africa in general.

PASA continues to invest in our veterinarians and their needs, with an ongoing commitment to quality training, scientific collaborations and conservation. The level of dedication and professionalism of the PASA veterinarians makes us all extremely proud. Our thanks once again go to the multitude of helpers with this project, including Lawrence Mugisha, Ambreen Magre, Owen Slater, Fabian Leendertz, Dominic Travis, Martine van Zijl Langout, Crispin Mahamba, Sandrine Mahe, John Kiyang, Lesa Longley, Rosa Garriga, Fred Nizeyimana, Ofir Drori, Michel Halbwax, Kay Farmer, Sheri Speede, Sue Walker and Andrea Fidgett.
Thank you also to the North of England Zoological Society (Chester Zoo) and the World Association of Zoos and Aquariums (WAZA) for assisstance in the production of this manual.

Steve Unwin, Doug Cress, Christelle Colin, Wendi Bailey and Wayne Boardman
November 2009

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SECTION 1

FORMULATING A VETERINARY HEALTH PROGRAMME

1.1 PASA VETERINARY MINIMUM STANDARDS (From the PASA Operations Manual)

S.Unwin, D Cress. Reviewer: PASA Steering Committee 2009

PASA Animal health policy

PASA member sanctuaries agree to provide the best possible health care to resident captive wildlife. Where possible, PASA member sanctuaries will employ/contract a veterinarian with primate health and disease experience, or arrange to have an off-site veterinarian available in an advisory capacity.

PASA member sanctuaries will ensure that at least one staff member is trained in primate emergency veterinary procedures (by a suitably qualified veterinarian) and is on site at all times.

PASA member sanctuaries will strive to abide by the PASA veterinary guidelines on primate health care as outlined in the PASA Operations Manual and PASA Primate Healthcare Manual (Boardman *et al*., 2004, Unwin *et al*., 2009)

PASA member sanctuaries will ensure that only primates that have cleared all quarantine/veterinary procedures are allowed to be moved to their permanent sanctuary/release site from which they may escape and/or come into contact with wild primates.

PASA member sanctuaries should ensure that all staff/volunteers/researchers/visitors that may have contact with the primates have complete health screens including vaccinations and annual TB testing.

PASA member sanctuaries should ensure that all staff/volunteers/researchers/visitors that may have contact with the primates have complete health screens as recommended by local Health Authorities and/or risk assessments of disease hazards (for example TB).

PASA Veterinary and human health and welfare Minimum Standards

PASA veterinary and human welfare goals:
Prevent disease entering the sanctuary;
Maintain health of animals within the sanctuary;
Prevent dissemination of disease to other institutions/populations.

To achieve these goals PASA members must secure the services of a veterinarian/s[1]. The veterinarian(s) must possess particular knowledge in the field of animal health as it applies to the species under his or her care.

[1] Whilst each sanctuary would ideally secure the resources to employ an experienced full-time veterinarian, it is acknowledged that this may not be presently possible. However, access to remote and/or consultancy services of a suitably qualified veterinarian should be within the remit of all PASA accredited sanctuaries.

Members must provide evidence of a preventative health programme for the sanctuary and any release programme. This programme will highlight diseases of concern and:

- Control and mitigate effects of endemic disease by disease surveillance and biosecurity;
- Minimise risk of imported disease by quarantine and pre import testing protocols;
- Prepare for exotic disease importation by effective disease contingency planning.

The role of the veterinarian/ veterinarian team, in consultation with sanctuary management, is to establish and maintain this health programme which must include the following components:

- Veterinary input into enclosure design to minimise disease spread and allow effective veterinary intervention as appropriate;
- Detailed animal records in a transferable format;
- A nutritional programme to minimise health and welfare issues;
- A safe and effective programme for the control of pests;
- Quarantine – newly arrived animals to be kept isolated as long as is necessary to ensure proper examination and disease screening before introduction to other animals at the sanctuary;
- An opportunistic disease screening programme – routine examinations, including parasite checks and TB tests, and preventative medicine including vaccination and endocrinological studies to be conducted at such intervals as recommended by the veterinarian/veterinarian team. All samples taken for further investigation must be documented, stored and shipped appropriately[2]. This programme must include a documented post mortem routine:

    - ✓ Dead animals to be handled in a way which avoids the risk of transmission of infection;
    - ✓ Post mortems to be carried out on all sanctuary animal deaths by the vet or a pathologist with relevant experience and training;
    - ✓ Following post mortem examinations carcasses and organs to be removed swiftly and disposed of safely;
    - ✓ Samples taken from post mortems must be documented, stored and shipped appropriately.

- Disease contingency plans for high risk diseases must be in place.

Members must maintain proper standards of hygiene, both in respect of the personal hygiene of the staff and that of the animal enclosures, food storage and preparation rooms, and treatment rooms.

[2] Refer to the PASA Health Care Manual (Unwin *et al*., in prep) for details on the collection, storage and shipment of samples.

Hygiene Aims:

- To minimise disease spread between sanctuary animals and staff and visitors;
- To monitor health of staff and volunteers (by consent);
- To communicate closely with local health authorities regarding local health issues and recommendations for staff disease surveillance and medical requirements.

Members must give due attention to the health and safety of staff and visitors by:

- Making continued efforts to resolve any potential threats to the safety of staff or visitors, along with complying with all statutory requirements;
- Having a written safety and emergency plan that is regularly reviewed and updated;
- Making sure all animal drugs, vaccines and other restricted veterinary products are kept safely under lock and key with access by authorised persons only;
- Except under the direction of the veterinarian, sanctuary personnel do not possess or administer controlled drugs.

1.2 PASA VET BEST PRACTICE and PREVENTATIVE HEALTH PROGRAMME OVERVIEW

S.Unwin (Based on various templates – see references) Reviewer: PASA steering committee 2009

1.2.1 Introduction

This section provides best practice guidelines for the medical management of primates in PASA member sanctuaries. Information is expanded on, and examples are supplied where relevant, throughout the remainder of this PASA veterinary healthcare Manual. The guidelines should facilitate the movement of animals into and out of sanctuaries without major health risks.
A veterinarian in charge of non-human primate healthcare will find a lot of information in a wide spectrum of veterinary literature. The PASA Veterinary Healthcare Manual remains the only sanctuary-specific veterinary manual, and should be regarded as a primary text. Online resources, including the PASA veterinary list-serve are also available. The Transmissible Disease Handbook[3] of the IDWG (Infectious Disease Working Group) of the EAZWV (European Association of Zoo and Wildlife Veterinarians) might be a valuable tool for diagnosis and therapy. Additionally, the O.I.E. International Animal Health Code (Zoonoses transmissible from non-human primates, 1999) and the Great Ape Health Monitoring Unit, based at the Robert Koch Institute in Berlin, can be consulted on current known zoonotic disease threats. Reference should also be made to the IUCN Guidelines for Nonhuman Primate Reintroductions (Baker, 2002) and the IUCN Great Ape Reintroduction Guidelines (Beck *et al*., 2007), and the sanctuary specific PASA disease of concern spreadsheets online through the list-serve, and attached as an appendix to this manual.

These guidelines, and the veterinary manual they are a part of, are for the use of PASA member sanctuary staff involved in the management of primates. They are not intended to be a do-it-yourself guide for solving medical problems in animals by inexperienced or non-veterinary staff.

PASA guidelines on minimum standards state that each sanctuary must secure the services of a veterinarian. The veterinarian must possess particular knowledge in the field of animal health as it applies to the species under their care. This means he/she must update their knowledge regularly, including information about relevant health regulations.

Numerous pathogenic micro-organisms can cause diseases in both human and non-

[3] The Transmissible Disease Handbook was developed by veterinarians working in European Zoos on diseases of concern. Each disease entry contains information on diagnosis including a list of laboratories in Europe that will conduct the test. It is updated every 5 years (last version 2008)

human primates, thus the potential will always exist for the transmission of disease between the two groups. Furthermore, pathogenic organisms can be inadvertently transferred between different groups of primates by staff working with more than one group. The veterinarian in charge has to cooperate closely with the staff, sanctuary management, local human healthcare workers and the veterinary and medical authorities. In some situations they may need a close cooperation with medical specialists. The veterinarian carries considerable responsibility for the well-being of the primates and staff, and often even for public health.

Infections and infestations shared by humans and other animals are often designated zoonoses[4], and non-human primates have always been noted for their zoonotic potential. All primates should be treated as potential carriers of zoonotic diseases. Similarly, all staff should be considered as a potential source of infection for the animals. Additionally it should not be underestimated that diseases might be spread by sanctuary visitors if the enclosure design or the management allows direct or indirect contact.

Diseases can be spread between non-human primates and humans by numerous methods, including physical contact (bites, scratches, exposure to excreted material), ingestion and airborne or aerosol transmission. A comprehensive list of specific zoonotic diseases will not be given; according to Brack (1987) there are several hundred. Up to date details of the most relevant diseases in African primates can be found in the following sections of this manual.

Sanctuary staff that care directly for primates must assume a large amount of responsibility for implementing the guidelines described, however, cooperation is also required from the managers in providing adequate equipment, facilities, staff, support and training.

The recommendations contained in this chapter are intended as guidelines only. Each PASA sanctuary member should develop its own written set of instructions for veterinary, husbandry and field staff, modifying the guidelines where necessary to take local circumstances and practices into account. This process should be carried out in cooperation with the sanctuary veterinary advisor, and the resulting document should be read and signed by all staff concerned.

1.2.2 The role of sanctuary veterinary staff:

- Routine inspections of the animals;
- Directing or carrying out treatment of all sick animals;
- Administration of vaccines, worming and other aspects of preventative medicine;
- Health monitoring of animals including laboratory examination of samples (via a submission process or in-house);
- Safe and proper collection and preparation of diagnostic and other samples;

[4] A zoonosis or zoonose is any infectious disease that is able to be transmitted (in some instances, by a vector) from other animals, both wild and domestic, to humans or from humans to animals (the latter is sometimes called reverse zoonosis)

- Training of sanctuary personnel in health, welfare and hygiene;
- Ensuring that post mortem examinations of animals are carried out on all deaths;
- Supervision of quarantine premises;
- The establishment of written procedures to be followed in the event of the accidental use of dangerous drugs;
- In addition, veterinary staff are to be involved in/consulted on:
  - ✓ The nutrition and design of diets;
  - ✓ Planning and enclosure design;
  - ✓ Release programme design and post release monitoring.

1.2.3 Veterinary Care of Non-Human Primates in PASA Sanctuaries

1.2.3.1 General health

Animals that are in good general health are far less likely to carry or suffer from infectious diseases than those living on impoverished diets or in suboptimal physical or social conditions. Constant attention must therefore be paid to good husbandry practice. Good welfare stems from making sure the animals have access to five basic freedoms thus:

*PROVISION OF FOOD AND WATER***:** Both food and water are basic needs. The method of food presentation, the frequency of feeds and the nutritional balance must be taken into account. Food should be presented in a manner and frequency commensurate with the natural behaviour of the species, as well as its nutritional requirements, which may vary according to season.

*PROVISION OF A SUITABLE ENVIRONMENT***:** An environment consistent with species requirements must be provided. This should include shelter from rain, heat, cold and shade as appropriate. For example, climbing species must be provided with appropriate three dimensional environments. A balance must be struck between hygiene and the species' biological requirements.

*PROVISION OF ANIMAL HEALTHCARE***:** (a) Injury: The provision of an enclosure designed to minimise the risk of injury is required. The design should allow animals to get away from each other. In mixed species' exhibits, care should be taken that one species cannot injure another. Enclosures should be designed to minimise the risk of predators entering the exhibit. (b) Disease: Curative and preventive veterinary medicine should be provided. Every effort must be made to provide a correct diet and suitably hygienic environment from which pathogens are excluded or controlled.

*PROVISION OF AN OPPORTUNITY TO EXPRESS MOST NORMAL BEHAVIOUR***.** Animals should be allowed the opportunity to express most normal behaviour, taking into account current enrichment and husbandry guidelines.

*PROVISION OF PROTECTION FROM FEAR AND DISTRESS***.** Particular areas to look at are: group composition, sex ratios and numbers of animals in an enclosure and space and furniture in both indoor and outdoor areas. Enclosure design

should allow for as much normal behaviour as possible, and provide areas of escape from other animals. Animals often benefit from mixed species environments. However, inter-species conflict can cause stress and this needs to be monitored, recorded and reviewed, including safety from potential predators.

An example of how general health can be maintained in a sanctuary is provided on the next page. **It is recommended each sanctuary fill this section out to make it specific to their situation, and use that as the executive summary of their entire preventative health programme.**

An integrated preventative health system aims to highlight diseases of concern and thus to:

- *Control and mitigate effects of Endemic Disease– by disease surveillance and biosecurity;*
- *Minimise risk of Imported Disease – by Quarantine/ pre import protocols;*
- *Prepare for Exotic Disease importation – by effective disease contingency planning.*

Article I. *Sanctuary: _____*

Article II. *Infectious Disease Surveillance Programme (to be completed by each sanctuary)*

(a) Who provides veterinary care in your sanctuary?:

- e.g Veterinary care for us is provided by 1 full time local vet who has an internationally recognised veterinary degree. The Government has been informed of our veterinary staff via the CVO (Chief Veterinary officer) of the country (or appropriate Governmental body that the Sanctuary operates under such as a Department for Forestry and Environment). He/she is fully aware of the requirements to report any suspicion of diseases of national importance to the CVO and a series of international veterinary advisors

NAMES and AFFILIATIONS:

DATE:

(b) Recognition of Infectious Disease within the sanctuary (Example):

- Keepers/ animal care staff undertake daily observations of all animals in their charge. (monitoring of animals by researchers in field based sanctuaries)
- Keepers/ animal care staff notify the veterinary department/ vet promptly of any suspicion of disease. (or vet checks each animal daily)
- The veterinary department investigates each report as soon as possible (according to priority) and will take further samples for investigation as appropriate.
- Keepers notify the veterinary department promptly of any deaths, including still births.
- The veterinary department will either perform a post mortem examination or arrange for one to be performed by trained veterinary pathologists.
- Where infectious disease is suspected relevant samples will be taken for further analysis.
- Opportunistic sampling and screening for diseases of concern (in both live and dead animals) is undertaken according to the attached protocol.
- Pre-release screening for diseases of concern.

(c) Preventing dissemination of Infectious disease (Example):

- Measures taken will be dependant on the type of infectious disease suspected. Where there is a strong suspicion of a disease of national importance protective measures will be put into effect and the CVO will be notified immediately.
- All animals are treated promptly to reduce the chance of them disseminating infections.
- Hygienic measures (eg foot baths, cleaning of equipment that must be shared between enclosures) appropriate to the type of infection suspected are put into place to reduce dissemination of disease.
- Infected animals and their in contacts are isolated from unaffected animals.
- Vaccination or prophylactic treatment will also be considered dependant on the disease concerned.

(d) Other preventative measures (Example):

- Prophylactic treatments (e.g. vaccination, worming – see relevant protocols)
- Pre-import screening / review of disease history of incoming animals – where possible
- Quarantine / isolation of incoming animals. (see quarantine protocols)
- Screening (+/- prophylaxis) for diseases of concern during isolation period. (see quarantine protocols)

(e) Audit (Example)

- Daily records kept of keeper observations. Medical records updated as required.
- Weekly review of ongoing clinical cases and post-mortem examination.
- Quarterly review of causes of morbidity and mortality.
- Annual review of preventative health care plan.

1.2.3.2 Veterinary Aspects of Enclosure Design

Aim: To provide enclosures that reduce requirements for veterinary intervention, but allow easy access if intervention is required.

Enclosure design should minimize direct or indirect contact between different primate species, keepers and visitors. All enclosures should provide adequate shelter to protect the animals from strong sun and heavy rain, as appropriate. All enclosures should include a double door system for staff entry, to reduce the risk of escapes. Often overlooked in the construction of indoor areas is the provision of adequate ventilation in indoor areas. The recommended number of air changes per hour should depend on the size of the inside enclosures, the local climatic conditions, the frequency of use of the outside areas and the total number of primates kept. Refer to Section 5.3.2 'Captive Primate Housing and Husbandry' of the PASA Operations Manual for more information.

Particular attention should be paid to eliminate sharp edges from the inside and outside enclosures, i.e. those edges on which animals and staff could cut themselves. Service passages must be sufficiently wide to prevent animals from reaching out to scratch or otherwise injure the keeping staff. Inside enclosures should be designed so that apes can be easily darted/ accessed by the veterinarian without any obstacles. Refer to Section 5.3.2 Captive Primate Housing and Husbandry section of the PASA Operations Manual, and later sections of this manual for more details.

1.2.3.3 Identification and Records

Aim: To provide an accurate record of the veterinary management of the sanctuary

Individual animal identification allows tracking of the animal and correlation with its medical records. All primates should be readily and reliably identifiable by the use of transponders and individual record numbers +/-tattoos/ photographs, and medical records kept up to date. All sanctuaries should aim to work with the same record keeping protocols/ software (e.g., a software package flexible enough to take into account the varying sanctuary setups such as ZIMS (Zoo Information Management System, being developed by ISIS (International Species Information System) as this will allow better collation of information across the network.

1.2.3.4 Veterinary Aspects of Nutrition

Aim: To minimise nutritional disorders by providing a nutritionally balanced diet to all animals within the sanctuary

Each sanctuary should produce and maintain up to date records on the nutritional requirements for stage of animal life of the species under their care, with reference to malnutrition/ juvenile/ pregnancy/. This information should then lead to a recorded current feeding plan and the whole combined in a diet manual.

Well formulated diets are vital for good health. Wherever possible, natural diets should be provided. However, if this is not possible, the diet should be based on nutritional content, rather than attempting to find 'similar' food items. Nutritional deficiencies may cause overt disease e.g. Metabolic Bone Disease

Diets should be evaluated regularly. It will be necessary to analyze the feed and the feeding practice to ensure that animals are actually eating what is being fed (i.e., not preferentially taking certain items). The diet can be nutritionally analysed to monitor for deficiencies. Nutritional deficiencies can also be monitored for diagnostically in blood (e.g serum vitamin levels, haematology and blood chemistry). Radiography, to measure bone density, is a useful adjunct test where possible.

Feed should be monitored for potential poisons. It is important to check an animal's enclosure for toxic plants (especially a new enclosure)

Use food also as an enrichment device to minimize stereotypic behaviour and improve psychological wellbeing. However, this sort of enrichment should not impinge on the nutritional requirements of the animal, neither should they be vital to them.

1.2.3.5 Pest Control

Error!

Aim: To minimise disease spread by controlling vectors and animals that spread disease within the sanctuary

Many infectious diseases of primates can be carried by invertebrate and vertebrate pest species frequently encountered in and around primate facilities. Specialist advice should be sought to reduce or eliminate such pests, which include ticks, insects such as cockroaches, snails, rodents and birds. This can be especially challenging in enclosures with natural vegetation, ponds and moats which may require constant attention in this respect. Organisms such as Encephalomyocarditis Virus (EMCV), *Shigella*, *Salmonella*, *Campylobacter*,

Chlamydia, *Leptospira*, *Yersinia* and even nematodes such as *Angiostrongylus* and *Capillaria* can all be introduced or spread by pest species.

Roaming domesticated and free-ranging wild species should be discouraged from entering the sanctuary where practicable. Domestic poultry, hoofstock and dogs in particular are a potential disease threat. Under no circumstances should domestic species be farmed or housed within the sanctuary property.

All food scraps should be cleaned up regularly during the day from enclosures and food preparation areas and soiled food should be disposed of correctly and not left for rodents to eat.

Precautions must be taken to guarantee that the control measures implemented do not themselves cause harm to the animals.

1.2.3.6 Quarantine (also refer to section 3.4, and others as mentioned in text)

Aim: to prevent importation of disease into the sanctuary. This is to prevent the introduction of new diseases to animals in captivity and from captive animals to the free-living population and to prevent possible infection of staff

Effective quarantine is the cornerstone of a preventative medicine programme. As such the length of quarantine is dictated by the diseases of concern. Three months (90 days) is often cited as a minimum to allow the incubation/ investigation of these diseases of concern to take place. All primates entering a collection, **irrespective of their origin**, should undergo a period of quarantine. During this period a variety of screening tests can be performed to establish their health status, their vaccination status can be reviewed, and a serum bank established for each individual.

With the exception of unweaned individuals being hand-reared , direct handling of conscious animals during quarantine should be avoided. In the case of animals requiring hand rearing, where possible there should be one caregiver per individual, and that person should not have contact with the animals within the sanctuary. The animal being hand reared should be placed with conspecifics within quarantine as soon as is possible, to help with the socialisation process.

Prior to and during the quarantine period the following procedures are advised:
In all cases personnel involved should have specific experience of working with primates and assisting in handling for pre-shipment and health management. Isolation should be in a separate building with separate air flow if possible and drainage and as far way from the other animals as practical – minimum 20m. Animals confirmed or suspected of having contagious diseases must be kept isolated. Staff should enter isolation area last, preferably at the end of the day, to prevent spread of infectious organisms, or there should be dedicated staff for this

area. They should wear protective clothing such as gloves and masks. Footbaths with disinfectant /separate footwear should be used. Strict hygiene procedures should be followed. Clean and disinfect area thoroughly when an animal leaves including food bowls and other items that were in enclosure. If animals of the same phylogenetic order as the infected animal are housed together, they too should be considered contagious. Keep domestic animals away from wildlife. During the defined isolation period all primates should undergo a health monitoring schedule which includes

Incoming Confiscations:

1. Basic information about the individual(s) should be supplied including area of origin, the animal's weight, temperamental characteristics, whether mother or hand-reared, current diet, and any physical abnormalities even if not a clinical entity.
2. As well as information regarding the health status of the animals to be moved, any significant evidence of recent zoonotic disease occurring in in-contact people within the previous six months should be notified.

Import/ Export between sanctuaries or other organizations:

1. Because of the risk of zoonotic disease both sanctuaries involved should review their risk assessments prior to the movement of the animals and implement protocols for management of the species involved which may be required or requested by the partner sanctuary.
2. Full I.D details including description, age, sex, distinguishing characteristics, microchip number and location and in some cases photographic I.D. should be supplied.
3. Basic information about the individual(s) should be supplied including the animal's weight, temperamental characteristics, whether mother or hand-reared, current diet, and any physical abnormalities even if not a clinical entity.
4. As well as information regarding the health status of the animals to be moved, any significant evidence of recent zoonotic disease occurring in staff working in the sanctuary environment within the previous six months should be notified to the sanctuaries involved.
5. Full clinical details should be supplied to the recipient sanctuary preferably 21 days prior to shipment. This must include any current treatment or medication. Any significant diseases occurring within the supplying collection within the previous six months should be notified to the recipient collection.
6. Current or past usage of any form of contraception and vaccination should also be advised including the length of time used and interval of repetition together with any noted side effects even if anecdotal.

Import/ export between field site and sanctuary:

1. As well as information regarding the health status of the animals to be moved, any significant evidence of recent zoonotic disease occurring in staff working in the sanctuary or field site environment within the previous six months should be notified to the sanctuary/ field site involved.

2. Pre release quarantine. Refer to the IUCN guidelines for reintroduction (Baker, 2002; Beck *et al*., 2007);
3. Procedures during quarantine. Refer to the IUCN guidelines for reintroduction (Baker, 2002; Beck *et al*., 2007).

Testing procedures:
A range of tests for disease agents and bio-parameters should be carried out during quarantine. It is recommended that the non invasive aspects of this testing (e.g., parasitology) is initiated as soon as practical. Testing requiring general anaesthetic should be delayed for 7 to 14 days, depending on the animal history and physiological and psychological condition on arrival.

- Full clinical examination under a general anaesthetic, including a careful assessment of weight, teeth, eyes, reproductive organs and identification (microchip).
- TB testing: refer to section 4.3 and 5.10 for up to date recommendations on tests (e.g., intradermal skin testing, PCR, Rapid tests, MAPIA, culture). Intradermal palpebral test for T.B. using MOT and/or bovine + avium tuberculin. NB. If using the intradermal skin test, any reaction is to be considered positive (refer to TB chapter on assessing skin tests). (Note: OIE recommends two tests within 30 days of export, although this may increase the risk of false positive reactions to the second test) It is recommended this be increased to 60 days (i.e., 2 TB tests within quarantine a minimum of 60 days apart).
- A minimum of 3 faecal screens, spaced evenly through the quarantine period for parasitological and bacteriological diseases of concern (see example chart below).
- Radiography if feasible, to check for such conditions as metabolic bone disease and air sacculitis.
- Haematology and blood biochemistry profiles.
- Serological tests for relevant diseases of concern (see example chart). **Other pathogens may be added to this list as our knowledge of their significance advances. A wider range of viral screening tests is generally appropriate for wild-born animals.**
- Banking of genetic material (see Section 3.17 for sampling techniques).
- Endocrine assessment (stress and reproductive hormonal analysis) - See section 3.16 and 3.17 for sampling techniques.

For additional information (refer to the relevant sections of the PASA Vet Manual for details):
Parasitology (3.10. 3.11, 3.17): Assessment of internal and external parasite burden. Repeated faecal tests (recommend formyl ether concentration technique, but other methods may be used such as flotation, sedimentation, Baermann-Wetzel method, culture) will be necessary to determine whether internal gut parasites are present. Even if those tests are negative and the animal has been treated it might be a carrier of *Strongyloides* due to hibernating larvae. Eggs of *Enterobius* will not be found by the above methods, but can be diagnosed by use of a scotch tape applied to the anus/perineum. *Capillaria hepatica* and *Angiostrongylus cantonensis* might only be diagnosed by serology or even post mortem.

Repeated testing will maximize the chances of detecting and identifying the more delicate protozoan parasites (*Amoeba, Balantidium, Lamblia, Blastocystis hominis, Dientamoeba histolytica*).

*Bacteriology (*3.17, 5.5*)***:** Faecal samples should be tested for the presence of pathogenic bacteria such as *Campylobacter*, *Shigella, Salmonella* or *Yersinia* species. Some of these organisms are only shed intermittently, necessitating the examination of several samples. It is recommended to collect only fresh samples and to use appropriate transport medium. Where pathogenic parasites or bacteria are detected, appropriate treatment should be given and its effectiveness confirmed by further tests during the quarantine period.

TB-testing (4.3, 5.10)**:** Very few individual TB tests are completely diagnostic and further veterinary advice should be taken for each set of circumstances, especially taking into consideration the animal's origin. Serological tests such as multiple antibody Rapid Test, complement fixation and PCR are other possibilities. In the case of a positive intradermal tuberculin test a range of other screens should be considered including the microscopic examination of sputum, radiography of the lungs, culture of material recovered from bronchial washings and gastric lavage, CT and differentiation of acid-fast bacilli by PCR. Infection with non-pathogenic atypical *Mycobacteria* may cause a positive tuberculin test.

Table 1: Example of Diseases of concern spreadsheet for pre import or quarantine sampling

Disease of Concern	Species	Justification	HAZARD (H/M/L)	LIKELYHOOD (H/M/L)	SHOW STOPPER? (Y/N)	Screening Test available	Type of sample required	Notes (eg sampling regime, vaccine available / recommended?)
TUBERCULOSIS caused by M. bovis or tuberculosis	All	Important cause of mortality/morbidity and ZOONOSIS	H	M	Y	Y – skin test, also gamma interferon blood test for some species Culture gold standard but slow and insensitive Investigating TB antibody Stat-Paks/ MAPIA from Chembio	Standard: Skin test ungulates, primates, Tracheal/ bronchial wash for culture Serum/ plasma (for stat pak)	Tuberculosis testing is a rapidly changing field. Keep up to date with the latest developments
Enteric protozoa (highlighting Entamoeba.histolytica, Balantidium.coli, Blastocystis.hominis, Dientamoeba.fragilis, Giardia)	Potentially all. Especially Apes	Most zoonoses. Confirmed clinical disease and carrier states in majority of NHP. Severe under reporting of protozoal infections in UK zoos suspected	H	H	N	Fresh stained faecal smear. Forminalised sample to check for oocysts. Fresh-frozen faeces for E.histolytica for PCR if available	Faeces	Single sample, 1 week apart. Important to note quality of faeces. If no in house ability, can put faeces in formalin for lab analysis of any cysts.
Enteric nematodes (highlighting Strongyloides, pinworm and Oesophagostomum)	All	Zoonoses. Known to cause morbidity in NHP's	H	M	N	Faecal parasitology -3 day pooled sample. Strongyloides may require charcoal culture for ID	Faeces	3 day pooled sample (plus previous clinical history of this being done if available)
Enteric bacteria (Highlighting Shigella (apes), Salmonella, Campylobacter, Yersinia.	All	Zoonoses – known to cause morbidity and occasionally mortality in NHP's	H	M	N	Bacteriology	Faeces	Salmonella should be typed and Campylobacter speciated, especially in subclinical carriers
Pneumonic bacteria – e.g Streptococcus pneumoniae	Apes and guenons	Carrier state common in humans and in NHP's. Confirmed deaths in apes and guenons)	M	H	N	Bacteriology	Pharyngeal swab	Known primate deaths in PASA sanctuaries
Virology (Severe) Ebola, Rabies, EMCV, Human MPV, Avian Influenza	All	Ebola and Rabies – severe zoonoses. EMCV potentially. Human MPV confirmed in apes	H	L	Ebola and Avian Influenza – Y, others potentially	Virus Isolation/ PCR/ ELISA etc. Quarantine time and history most likely for ebola	Serum. Throat swab for Human MPV.	Contact Fabian Leendertz at GAHMU for assistance
Virology (Moderate) RSV, Hepatitis B, Measles, SIV, STLV, polio, yellow fever	Apes (SIV/ STLV all)	Hepatitides mostly subclinical. Potential for cross species infection	H	H	N	Virus Isolation/ PCR/ ELISA etc.	Serum	Contact Fabian Leendertz at GAHMU for assistance
Anthrax	All	Severe zoonosis	H	M	Y	Distinctive staining, culture and PCR	Blood	Contact Fabian Leendertz at GAHMU for assistance

1.2.3.7 Ongoing Preventive Medicine Programme: Annual Disease Surveillance Plan (Section 3.5)

Aim: To minimise circulating disease within the sanctuary by designing an effective audited disease surveillance programme

The veterinarian must design and implement an annual disease surveillance plan which is subject to annual internal audits, and PASA audits as determined by the PASA Steering Committee.
This might include:

- Daily written reports about the health of all animals within the sanctuary including any symptoms of disease, anomalous behaviour, births, deaths, veterinary treatments, etc.
- Regular faecal testing for pathogenic bacteria and parasites every six months - more frequently if a particular problem is known to exist. Where an infection with pathogenic bacteria or parasites has been treated, follow up faecal samples should be examined to establish the effectiveness of treatment.
- If there is a vaccination programme, the vaccine status of each animal should be reviewed annually and boosters given when appropriate.
- Serum samples should be collected and stored at minus 70 ^{0}C or below as and when the opportunity arises. Serum banks thus created can be invaluable in the diagnosis of viral disease, the determination of vaccine efficacy and in the screening for new diseases as they are described. Furthermore serum banks represent an invaluable research tool.
- Periodic (e.g., every 2 years) physical examinations are very useful to assess the health status of the animals. If primates are immobilized for any reason, blood samples should be collected for haematology, serum biochemistry and serology, urine should be collected for a urinalysis and a TB test performed
- Comprehensive post mortem examination of all apes dying within the collection (See Section 3.18).

1.2.3.7.1 AN EXAMPLE OF OPPORTUNISTIC HEALTH SCREENING

Apes

Diseases of Concern	Hep A, Hep B, SIV, Mycobacteria tuberculosis/ bovis/ avium-intracellulae, Shigella, Campylobacter, Salmonella, Entamoeba histolytica, Balantidium coli, Enterobius vermicularis, Strongyloides spp., other enteric nematodes.
Samples Required	Blood (10mL 8mL Plain, 2 mL EDTA. Keep cells for genetics), fresh faeces, tracheal wash. Hair inc. root for genetics.
Tests Required	Viral Serology, Haematology, Serum biochemistry, faecal culture, faecal wet prep (within 1-2 hours of collection), faecal float (parasitology), ID TB Test
Routine treatments	Tetanus vaccine opportunistically – once confers immunity for 5-10 years
Ongoing monitoring	6 monthly faecal checks – treatment as appropriate.

Primates - simians

Diseases of concern	Herpes B (colobus only) ,(monkeypox, SIV – both on initial presentation), Mycobacteria, Shigella, Campylobacter, Salmonella, Yersiniosis, protozoal and nematode parasites
Samples required	Blood (1-5mL Plain, 0.5 mL EDTA), fresh faeces, tracheal wash, anal sellotape test.
Tests required	Viral Serology (Herpes only), Haematology, Serum biochemistry, faecal culture, faecal wet prep if possible (within 1-2 hours of collection), faecal float (parasitology), ID TB test
Routine treatments	Tetanus vaccine mandrills opportunistically – once confers immunity for 5-10 years.
On going monitoring	Annual faecal checks – treatment as appropriate.

1.2.3.7.2 Vaccinations (Section 3.12)

A sanctuary vaccination regime will be dictated by what diseases are found in the local area, what diseases are found within the sanctuary, what diseases can be vaccinated for, and what the destiny for the animals in question is. For example, it will usually be inappropriate (but not always) to vaccinate animals due for release.

Tetanus: Clinical tetanus has been reported in wild and captive primates, and is generally fatal. Three intra-muscular doses of tetanus vaccine (standard human tetanus toxoid containing 40iu of tetanus toxoid per dose is acceptable) are given at 2 to 3 month intervals, starting at 3 months of age. Intramuscular boosters are given after 5 years and at 10-year intervals thereafter.

Measles: All apes are susceptible. Apes should be given a single dose at 15 months of age or over. Live measles vaccine should not be given at the same time as other vaccines, or to animals with other infections, or to any immuno-suppressed animal. An intramuscular booster is given after 10 years.

Polio: Clinical poliomyelitis has been reported in chimpanzee, gorilla and orangutan. Three doses of live, oral, trivalent polio vaccine (containing attenuated strains of poliomyelitis virus, types 1, 2 & 3) are given at 1 month intervals. This may be started at 2 months of age (or earlier if a particularly high risk exists). Oral boosters are given after 5 years and at 10 year intervals thereafter. It is important to give the oral polio vaccine to all animals in a group at the same time. This is particularly important with the first dose of any course. An alternative strategy is to use the enhanced potency inactivated polio vaccine (eIPV) containing polio viruses of all three types inactivated by formaldehyde. Three 0.5 mls doses are given by sub-cutaneous injection at monthly intervals, starting at 2 months of age or above. Booster doses of eIPV are given after 5 years and at 10 year intervals thereafter.

Other diseases: Vaccine programmes should be adapted to changes in disease prevalence and increased knowledge of the efficacy and safety of available products. In unusual circumstances, i.e. in the face of specific challenges, primates can be vaccinated against influenza, bacterial meningitis, *Haemophilus influenzae, Pneumococcus,* viral hepatitis A and B. If apes are carriers of hepatitis B the offspring may be protected by vaccination immediately after birth.

It is not advised that apes are vaccinated against tuberculosis with BCG as the vaccine interferes with tuberculin skin tests for TB and probably only induces a limited period of immunity.

Do not use the triple vaccine known as DPT or DTP (diphtheria, tetanus and pertussis), or the typhoid vaccine. There have been a large number of adverse reactions recorded in non-human primates and apes are not particularly susceptible to diphtheria or pertussis.

Mumps and rubella (German measles) are primarily sub-clinical diseases in most non-human primates but might cause serious symptoms in apes.

As and when the opportunity arises, serum samples from vaccinated primates should be tested to establish the effectiveness of the vaccine schedules.

1.2.3.7.3 Endocrinology, Behaviour and Reproduction (Contraception) (Section 3.16)
Every sanctuary must have a reproduction policy. It is currently PASA's policy not to breed primates, unless they are an endangered species and there have been international recommendations for breeding in captivity. In these circumstances decisions are to be made on a case by case basis.

Hormonal analysis can be utilised monitoring stress levels (for example, before and during a pre-release period), as well as pairing sexual behaviour and secondary characteristics such as swellings with internal reproductive changes such as ovulation and pregnancy. Refer to the endocrinology and contraception sections of the PASA vet manual for details.

1.2.3.7.4 Parasite Control (Section 3.10)
Every sanctuary must have a parasite control policy, preferably via a test and treat protocol.

All primates in the sanctuary are by definition confined in comparison with their natural habitat. Consequently, there is a real possibility that parasites can become more significant and cause disease. Routine examination for endo and ectoparasites should be performed on arrival and at regular intervals. This is especially important for parasites with a direct life-cycle (e.g., roundworms) as large numbers of eggs can build up in the environment and infect subsequent cage occupants. Strategic use of anthelmintics will help to control certain parasites. Refer to the parasitology section of the PASA Vet Manual for details.

1.2.3.7.5 Post Mortem Examination (Section 3.18)
A thorough post-mortem examination should be carried out by a competent and experienced pathologist or veterinarian without unnecessary delay on all animals dying in a collection. Particular care should be taken with primates dying in quarantine as these animals must be assumed to be of high zoonotic potential until proven otherwise.

Even if the cause of death seems obvious a post mortem examination is strongly advised as valuable information about the health of a group of animals can be obtained. For example, a parasitological examination of internal organs, including histology of the duodenum for *Strongyloides*, liver for *Capillaria, Echinococcus, Amoeba,* lung for *Pneumonysses* and *Angiostrongylus etc.*, might give valuable hints to possible carriers.

1.2.3.8 Disease Contingency Planning (Section 3.2)
Examples of disease contingency planning for various diseases (completed by the delegates of the 2008 PASA veterinary workshop) relevant to PASA sanctuaries can be found in section 3.2 of this manual. They illustrate how the process can be undertaken, but will need to be modified and updated for each sanctuary situation. They are included as an example of how sanctuaries can begin to plan for a disease outbreak.

1.2.4 Healthcare of People Working in PASA Sanctuaries

Aims:

- *To minimise disease spread between sanctuary animals and staff and visitors*
- *To monitor health of staff and volunteers (by consent)*
- *To communicate closely with local health authorities regarding local health issues, and recommendations for staff disease surveillance and medical requirements*

Preventive medicine is also designed to ensure human safety and protect human health. PASA requires sanctuary managers to assess the risk of infection to employees and other people who may be working with the animals and their by-products. Where a risk is identified, appropriate preventative or control measures must be applied.

1.2.4.1 Pre-employment staff screening

To reduce the dangers of disease transmission to primates, prospective new staff members should undergo certain health checks, rather like the health checks carried out during quarantine for the animals. This pre-employment medical check has clear advantages for staff and employer alike, and should be developed in cooperation with the local health authority:

- New staff members should not have any contact with primates for the first two weeks of employment. This should allow sufficient time for the development of most infectious diseases that the new employee may be incubating when taken on, and for the completion of specific tests detailed below;
- Ideally the candidate should undergo a thorough medical examination by a doctor;
- Faecal tests should be conducted to establish whether the prospective staff member is carrying any pathogenic enteric bacteria or parasites;
- A skin test for tuberculosis should be carried out. If this is positive, the doctor will probably suggest further tests;
- A blood screen for hepatitis B and C should be carried out;
- Prospective staff should be offered HIV testing;
- The vaccine status of the new employee should be reviewed. It is important that vaccinations against hepatitis A, hepatitis B, tetanus, measles and polio are current.

These measures are suggested purely on medical and veterinary grounds. No comment or advice is given concerning the financial or legal implications of the tests or any treatment that may be required as a result.

1.2.4.2 Sanctuary Volunteer requirements

It is up to new volunteers to make sure they are up to date with relevant vaccinations, knowledge of the diseases in the area they are working, including malaria, and have taken all necessary precautions to minimise the risks of these diseases. It is also recommended that volunteers be fully medically insured. A

two week isolation period, as for new staff above, is recommended on arrival Somewhere, perhaps in management I think we need to make firmer recommendations to guide sanctuaries on requirements for recruitment of volunteers.

1.2.4.3 Health of staff during employment

Staff health should be monitored at least every 12 months;

- **It is essential that all staff members are in good general health. People that are run down in any way are far more likely to contract infectious diseases than healthy individuals;**
- Six-monthly faecal tests for pathogenic bacteria and parasites (may differ for each sanctuary depending on diseases of concern), are advised. Testing in instances of protracted diarrhoea (longer than 5 days) and/or provision of human approved anthelmintic every 3 months is also recommended.
- Each staff member should ensure that all vaccines are current; this requires monitoring by sanctuary managers to ensure compliance;
- An annual TB test is recommended. If this is positive, the doctor will probably suggest further tests;
- An annual HIV test is recommended. Positive staff members should have CD4/CD8 assessment every 3-6 months, and be provided with effective HAART medication when appropriate and possible.

1.2.4.4 Staff illness and injuries

Staff that are working and sick should at the discretion of the veterinarian be sent home or not allowed to work with susceptible animals. Colds, flu, measles, salmonellosis, viral hepatitis and many other infections can be passed to primates and may cause serious disease in an ape collection.

- All injuries, accidents and illnesses of staff should be recorded.
- Bites and scratches should be thoroughly washed (not scrubbed) and medical attention sought if severe. Further detailed information should be available to staff working with animals in quarantine and animals in the collection that have been incompletely screened.
- Staff members who are ill should not work with animals or prepare food.
- Staff with active herpes simplex lesions, should not work with primates and should be encouraged to seek medical advice about treatment. Staff with children or other family members suffering under infectious diseases like measles, German measles, mumps, chicken pox, scarlet, student kissing fever (mononucleosis, EBV) should not work with apes.
- If a doctor is consulted about illness in a staff member, he/she must be made aware that the patient's work involves care of non-human primates.

1.2.4.5 Staff personal hygiene

High standards of personal hygiene are required from primate keepers if the transmission of infectious zoonoses is to be avoided.
Frequent hand washing is probably the single most important measure to reduce or prevent the spread of infection. Washing is particularly important

immediately before and after working with any primates. Hands should always be washed after handling bedding and other enclosure materials, uneaten food, faeces, urine, blood, saliva and any other body secretions. Although disposable gloves should be worn when handling primates or primate material, hands should still be washed after gloves are discarded. In order that staff may wash effectively and sufficiently often, it is vital that suitable facilities are provided. These are best placed just outside animal holding areas;
It is best practice for animal staff to wear a range of protective clothing when working in primate facilities. Generally speaking this involves the use of overalls, rubber boots, and disposable gloves. Fully protective goggles and facemasks may also be necessary where a particularly high risk of zoonotic infection exists, such as when working with any primate in quarantine;
To reduce the risk of mechanical transmission of infectious agents between different primate facilities/houses, separate sets of protective clothing should be available for staff in each place. Work clothes should be washed in the primate unit or sent in sealed bags to a laundry and should not be taken home by staff for any reason;
Boots should be washed and preferably disinfected before entering and after leaving primate houses. Suitable facilities should be made available by the management;
People with open cuts or sores on their hands must wear disposable gloves when working with apes;
Staff should be encouraged to keep hands away from their face when working in animal areas. It is remarkable how often people touch their faces without thinking about it! Similarly, staff should be discouraged from putting pencils, pens etc. into their mouths;
No smoking, eating, drinking or spitting should be permitted in animal areas.

1.2.4.6 Enclosure cleaning

Protective clothing (overalls, boots, disposable gloves & masks, goggles) should be worn when cleaning animal areas. Protective cloth should be washed, cleaned and stored in the animal area;
Bedding and excreta should be removed in sealed bags to avoid the spread of material by the wind;
Animal areas should be cleared and scrubbed before hosing down. High-pressure hoses or steam cleaners should be avoided if at all possible, as they tend to create aerosols or sprays of potentially infectious material.

1.2.4.7 Equipment

Restraint equipment should always be in good working order. Nets, gloves, squeeze cages, crushes and crates should be regularly inspected. Defective equipment can lead to injuries to animal and man, and in the worst case escapes. Equipment must be cleaned after each job to avoid the mechanical transmission of infectious material. Needles, blowpipe darts and surgical instruments should be handled with extreme care as after use they might be contaminated with various bacteria and viruses.

1.2.4.8 Zoonotic infections

Staff should be made aware if primates in their care are known or suspected to be suffering from potentially zoonotic infections. Additional measures (if any) to prevent transmission of infection should be explained. This job will fall primarily to the veterinarian.

1.2.4.9 Pregnancy

Staff who are pregnant should not be working with primates and should seek specialist advice.

1.2.4.10 Immuno-suppressed staff

Staff who are immuno-suppressed for any reason should not be working with primates and should seek specialist advice.

1.2.4.11 Veterinarians

Many of the animals examined and treated by veterinarians will be sick and therefore the risk of zoonotic infection is often higher than for most animal care staff;
Veterinarians must adopt the most rigorous standards of personal hygiene and wear disposable protective clothing as often as is practical;
Particular attention should be paid to avoid the mechanical transmission of infective material via clothing and equipment, both between different primate houses and between different collections;
Veterinarians must ensure the correct disposal of clinical waste.

1.2.4.12 Other people

It should always be remembered that all other people who have access to the primates at the sanctuary may pose a threat to the animals and may themselves be at risk of infection. Therefore the role and management of volunteers, students, temporary staff, visiting zoo personnel, contractors working in animal areas, media personnel and in some cases, visitors, needs careful consideration.

PASA strongly urges that direct contact between sanctuary animals and non-staff personnel should be forbidden, whatever the justification. Under no circumstances should children be allowed to have direct contact with infant/juvenile primates.

1.2.4.13 Staff training

Caregivers are the eyes for the veterinarian, they are the ones who work with animals most of the day and who get to know their behaviours;
Training caregivers in observational skills and an understanding of the significance of certain changes in behaviours on the health of animals is an essential component of preventative medicine. They should have a good working knowledge of the important primate zoonoses;

Caregivers should be trained to act promptly to prevent an animal from becoming sicker and to avert injuries;
The relationship of the caregiver with their animals is important in reducing stress levels. Training of operant conditioning techniques is a significant advance in health monitoring and thus disease prevention;
Ongoing staff training should also include updated information on exotic diseases that may threaten the sanctuary, as well as any disease outbreak that may occur within the sanctuary.

1.2.5 Veterinary Research and Collaboration

Please refer to the PASA research policy. Veterinary research carried out in PASA sanctuaries MUST comply with this policy and be of a high scientific calibre. Veterinary research must demonstrate or have implications for our understanding of one or more of the following:

- Biodiversity conservation
- Species conservation
- Species ecology
- Animal health and welfare
- Species husbandry
- Contraception and reproductive health

Collaborative efforts with other PASA members, universities, conservation projects, Governments etc are actively encouraged.

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1.3 PREVENTATIVE HEALTH PROGRAMME OVERVIEW

S.Unwin and S.Sanderson

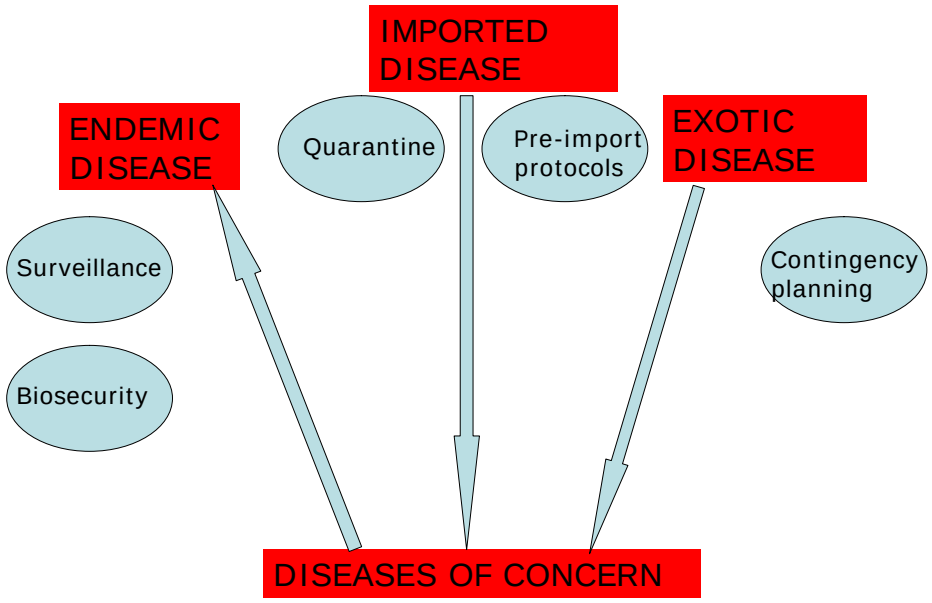

Figure 1. Management options for Diseases of Concern.

One of your primary roles as PASA vets is to be able to manage, control and minimise disease issues associated with your sanctuary. Figure 1 highlights procedures you can utilise to manage your diseases of concern in your sanctuary (blue circles). The main factors in controlling endemic diseases – those diseases that you already have in your sanctuaries, are surveillance for disease, usually by regular health monitoring, and biosecurity - procedures to reduce the chance of spreading those diseases further. Disease surveillance is also a useful tool as an early warning system for a potential epidemic. Well maintained records of this surveillance also provide a vital reference for disease investigation.

Quarantine, and potentially pre import protocols, helps minimise the risk if importing disease into your sanctuary, or exporting them from the sanctuary – be they diseases you know already exist and are trying to control, or those diseases that may be exotic. Contingency planning for exotic disease is a very useful process to prepare your sanctuary for an outbreak of a disease.

As the vet, or a member of the veterinary support team, you are one of the people responsible for the health and welfare of the animals in your sanctuary. It is a requirement that you work effectively with the sanctuary managers and other relevant staff to make sure the animals under your care are as free of disease as is possible. Therefore - what are your diseases of concern, and more

importantly, how have you concluded that these diseases are important issues? Use the box below to note down your answer.

What are you 'Diseases of Concern' for Your Sanctuary?
How have you decided that these are important? Why are they important?

Figure 2 illustrates areas of a preventative health programme already in place in PASA sanctuaries. Can you name all the components of a preventative health plan? – use the box provided on the next page. When you have completed this task, refer to the PASA veterinary guidelines and minimum standards in section 1, making notes where your sanctuary fails, meets, or exceeds the standards. This will provide you with the starting point of what you need to focus on for the disease management in your sanctuary. The following sections on risk analysis and epidemiology are designed to introduce you to tools that can help you make the decisions of what disease issues need to be investigated, and what procedures need to be introduced or refined. The following sections of this manual provide practical notes on combating disease issues you may encounter. However, never forget your colleagues as a source of advice if you need as well.

1

2

3

Figure 2. Aspects of a preventative Health Programme in a PASA sanctuaries – Emergency medicine and surgery in a trauma case, Tacugama Chimpanzee Sanctuary(1), opportunistic examination under anaesthetic, Limbe Wildlife Centre (2), diagnostic testing, Centre for Chimpanzee Conservation (3)

WHAT DOES QUARANTINE MEAN AT YOUR SANCTUARY? (Official Definition: A period of time during which an animal, person, or material suspected of carrying a contagious disease is detained under enforced isolation to prevent disease from entering an area. The term also refers to the place of such detention. Refer to the PASA Vet Guidelines.)

WHAT DOES BIOSECURITY MEAN AT YOUR SANCTUARY? (Official Definition: Precautions taken to minimize the risk of introducing an infectious disease into an animal population).

WHAT ARE THE COMPONENTS OF A PREVENTATIVE HEALTH PROGRAMME?

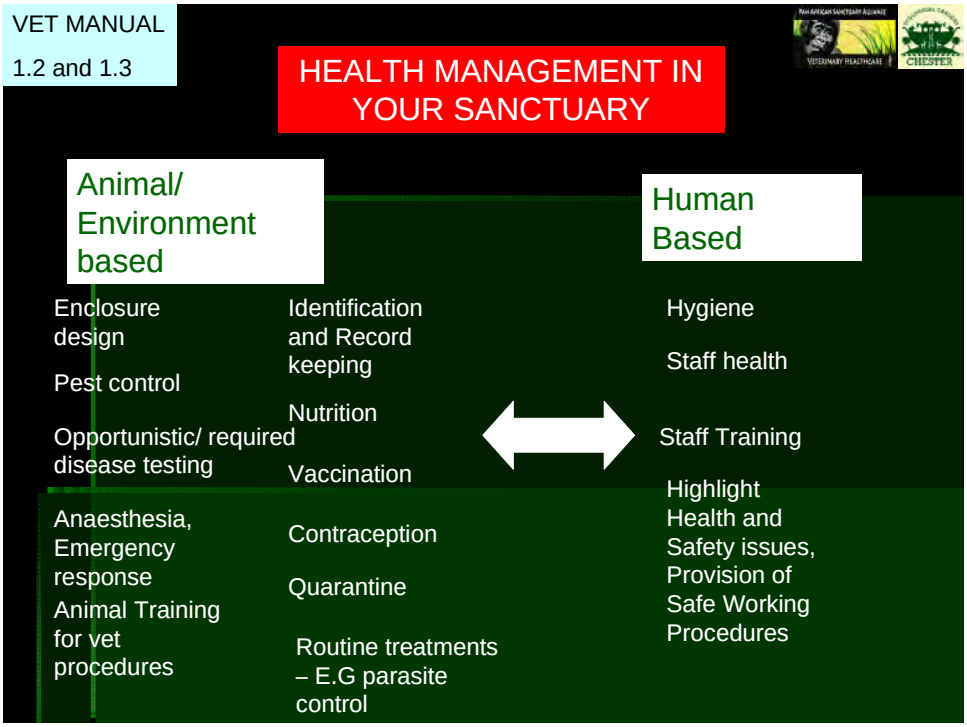

Figure 3. Components of a Preventative Health Programme

SECTION 2

SANCTUARY HUSBANDRY

&

MANAGEMENT

SECTION 2: SANCTUARY HUSBANDRY & MANAGEMENT **Page**

This section is designed as a checklist. Section 3 and 4 go into more detail for the majority of veterinary related issues.

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2.1 PRIMATE HOLDING FACILITY DESIGN

- Designed to promote species typical behaviour and development
- Provide sufficient space and environmental complexity to allow species typical sub-groups to form
- Constructed of materials that ensure animals will be contained at all time, and are safe for the animals, staff and the public
- Facilitate the effective maintenance of a clean, healthy environment

Spatial Requirements

- The size and shape should provide freedom of movement, both horizontally and vertically

- Each animal should be provided with sufficient space in all directions to enable it to:
    - Take exercise
    - To be protected from undue dominance and conflict
    - To be provided with its social and husbandry needs

Weather Protection

- Sufficient shelter must be provided to allow protection from
    - Wind
    - Rain
    - Sun
    - Extremes in temperature

Substrate and Drainage

- Must be well drained and the substrate predominantly comprised of inert material that is non-abrasive to the animals feet and impervious to water

- Drainage must quickly carry excess water away from the enclosure

- Unless it carries only surface water, an open drain must be inaccessible to the animals

Enclosure Furniture

- The enclosure must include where appropriate, such items as bedding material and branch work to aid and encourage normal behaviour

- No objects, furniture, apparatus, plants or other items that could interfere with the welfare of the animals or with efficient husbandry shall be kept, or allowed to remain in the enclosure

Gates and Doors

- The entrance (gates, doors, slides) must
    - Open inwards towards the enclosure
    - Be through successive gates or doors
    - Provide for safe means of access and a clear view

- Be designed so that an animal cannot
    - Lift the gate or door off its hinges or a slide off its tracks
    - Unfasten the security device

Exhibit and Space Rotation

This increases the behavioral enrichment and available space for intensively managed animals

QUARANTINE FACILITY DESIGN

- Must have a separate quarantine area with the ability to comfortably accommodate the species

- The primary goal is to ensure physical and spatial separation (minimum 20m separation)

- Quarantine facilities are usually composed of two physically separate areas
    - The staff area
    - The animal holding areas

Staff Area

- Storage space for cleaning equipment

- Rest room and a locker room with shower facilities for staff

- A single or double corridor system

- A footbath containing an effective disinfectant must be used prior to entering the quarantine facility

Animal Holding Areas

- The building must meet the requirements for any animal holding facility

- Anterooms should be attached to the animal holding room

- Indoor Housing of Animals
    - Lighting in indoor housing must be adequate
    - Indoor housing for an animal must be provided with ventilation
    - Cage size and design. Should be at least 1/3 the prescribed exhibit floor dimensions. The minimum prescribed height requirement must be provided. Should address security, safety and a means for

restraining the animal. A small number of large rooms prevent the capability of accepting new animals
    - Substrate and Drainage. The animal holding room floors, walls, and ceiling should be impervious to moisture to facilitate cleaning and disinfecting. The drainage system must be totally separate from regular holding areas

- Outdoors Area
    - Can be attached to the indoor area
    - Should be physically separated by walls which should be a minimum of 15 ft high
    - Should maximise the use of 3-D space

- Support facilities
    - There must be an area for carcass disposal
    - A separate garbage pit

VETERINARY FACILITY DESIGN

- Basic components include
    - Siting of facility: Proximity to animal enclosure; acoustic/visual separation. Good accessibility
    - Office area: Workspace, Records, Shelving
    - Clinical/Operating room: Operating table, Work benches (approx. 1m high), Equipment, Circulating space (1.2-2m), Ventilation, Lighting (Natural & artificial)
    - Floor and wall finishes Storage Facilities: Size and number of equipment e.g. Rooms, shelves, cabinets, Safety e.g. Lockable cabinets, Preservation e.g. Fridges
    - Specialised rooms/areas, Laboratory, X-ray, Post Mortem Room, Wash down area

2.2 CHECKLIST OF VETERINARY FACILITIES, EQUIPMENT AND SUPPLIES FOR PRIMATE SANCTUARIES

FACILITIES

- Examination room which is secure with good lighting and ventilation
- Examination table
- Sink and running water
- Bench space
- Secure drugs cabinet
- Stretcher

EQUIPMENT

- Weigh scales
- Boxes/ Crates/ Nets
- Gas anaesthetic machine – preferred
- Radiographic machine – optional
- Microscope and basic laboratory materials
- Darting equipment
    - dart pistol
    - darting syringes
    - box to hold needles, syringes and extras

- Antibiotics
    - Amoxicillin
    - Amoxicillin – clavulanic acid
    - Cephalexin
    - Ciprofloxacin
    - Doxycycline
    - Enrofloxacin
    - Erythromycin
    - Gentamicin
    - Metronidazole
    - Penicillin G benzathine
    - Trimethoprim – sulphadiazine

- Antiviral
    - Acyclovir

- Antifungals
    - Itraconazole
    - Ketconazole
    - Lufenuron

- Anthelmintics & Antiprotozoals
  - Albendazole
  - Clindamycin
  - Doxycycline
  - Fenbendazole
  - Ivermectin
  - Metronidazole
  - Paramomycin
  - Praziquantal
  - Pyrantel pamoate
  - Tinidazole
  - Secnidazole

- Antimalarials
  - Artemether
  - Mefloquine

- Anti-inflammatories and Analgesics
  - Acetaminophen
  - Dexamethasone
  - Flunixin
  - Ibuprofen
  - Prednisolone sodium succinate
  - Paracetamol
  - Flunixin
  - Buprenorphine

- Drugs for respiratory infections
  - Paediatric cough suspension for dry cough (which can contain anti-tussives) and wet productive cough (without an anti-tussive)
  - Coughing is a symptom – the cause must be addressed
  - Coughing is a positive reaction to help remove excessive mucus etc and so should not be suppressed when the cough is productive

- Gastro intestinal drugs
  - Antacids: Gaviscon, Maalox
  - Antiemetic: Metoclopramide
  - Anti diarrhoeal: Bismuth subsalicylate, Kaolin and pectate mixture
  - Laxative: Mineral oil (liquid paraffin)

- Topical drugs – skin, eye, ears
  - Antiviral : acyclovir
  - Bacteriostatic/bacteriocidal, antiseptic, fungistatic, scabicides and pediculicides, corticosteroids: numerous preparations should be locally available (in pharmacies). We recommend at least one preparation from each of these groups is included in basic supplies

- Ophthalmic drugs
  - Flourescein

  - Antibiotic drops / ointment (locally available)
  - Corticsteroid preparations (should only be used in the absence of active corneal damage – assessed with fluorescein)

- Rehydration fluids
  - Oral electrolyte solution (home made or commercial product)
  - Sterile lactated ringers solution for intra venous infusion
  - 0.95 saline for IV
  - Lactated ringers solution
  - Dextrose 5%

- Emergency drugs
  - Adrenaline
  - Atropine
  - Dexamethasone
  - Diazepam
  - Doxapram
  - Prednisolone sodium succinate

- Vaccinations
  - Tetanus
  - Polio
  - Hepatitis B
  - Measles

- Sedative / anaesthetics
  - Ketamine
  - Medetomidine
  - Atipamazole
  - Zoletil
  - Isolurane/ Oxygen/ Vapouriser/ anaesthetic machine

- Wound dressings

- Consumables
  - Syringes and needles
  - Giving sets
  - IV catheters
  - Tape/ Supa glue
  - Blood collection tubes
  - Faecal collection bottles

- Write dates of reconstiution on vials/bottles
- Range of antiseptics and disinfectants (Pevidine, Hibitane, chlorohexidine)
- Laryngoscope (VIP); Endotracheal tubes (various sizes)
- Autoclave and sterilizer; portable X-ray
- Pulse Oxcimeter, Capnograph

2.3 PROTOCOLS FOR CONFISCATION

GATHERING INFORMATION

- Individual (age, sex, temperament, any medical complications etc)
- Locality of the village/area with the individual i.e. distance
- Who, how & period the individual has been kept
- Check whether you might require special needs i.e. meds, permits, money etc

PLAN AHEAD

- Other possible recipients before taking on the responsibility
- Consider personnel for quarantine
- Documentation
- Transport
- Quarantine facility
- Equipment required for a confiscation

- Consider other recipients before accepting responsibility if
    - Lack a quarantine facility & personnel
    - Lack Documentation
    - Lack Transport/ Funds
    - Equipment required
- **Note: This may be temporary/permanent**
    - If the individual has an infectious condition that will jeopardize the livelihood of the resident chimpanzees
    - Temporary fostering by individuals with acclaimed experience with the species or the order (while still dealing with the other logistics)

Quarantine Preparation

- Consider working out a system if you have limited staff for resident & the quarantined
- Consider expertise for some individuals
- Presence of volunteers might come in handy
- Age, sex & character are important in selecting quarantine staff
- Disinfecting of facility

Documentation Needed

- International
    - *Letter of approval from Wildlife Dept.*
    - *Letter of from veterinary dept.*
    - *Import CITES permit*
    - *Export CITES permit*
    - *Full Health Exam with health certificate*
    - *Check IATA animal transport regulations*

- Local
    - *Letter from the local mandated organization*
    - *Personal IDs*
    - *Vehicle documents etc*

Quarantine

Ensure that all that is required for quarantine is available before the confiscation.

Such as, disinfection of the facility, bedding, food and a procurement system, quarantine protocol, personnel & notification of the veterinary department where applicable

Transport

- Vehicle (serviced, roadworthy, proper documents, big enough etc…..)
- Due to the usually limited personnel, the veterinarian should double as the driver (preferably)

Problems experienced in confiscation

- Bureaucracy
- Security problems
- Lack of cooperation by the source
- Distance and Locality
- Public attraction
- Social and behavioral disorders
- Medical state of the individual

Equipment required for a confiscation

- Vehicle Preferably a van
- Cage
- Towels
- Appropriate keeper
- Veterinarian
- Hot water bottles
- Note book
- Medical supplies
    - Fluids (LRS, ORS, Plasma)
    - Antibiotics
    - Surgical equipment
    - IV lines, NG tubes & spoons
    - Dietary supps i.e. K+, Vitamin K etc
    - Furesmide

2.4 NEW ARRIVALS PROCEDURE

CONSIDERATIONS

- New arrival procedures - first two weeks
- This is the period of stabilisation
- Primate goes into quarantine with the help of care givers
- Always act calmly and be quiet – minimal fuss
- Need to feel secure

EXAMINATION OF THE NEW ANIMAL - THIS MAY NEED TO BE QUICK

- Assess how alert the orphan is
- Assess level of dehydration
- Assess if any diarrhoea
- Assess if any breathing difficulties
- Assess if getting stressed
- Assess age – check teeth
- Check mucous membranes to see if anaemic
- Check if periorbital swelling/ large abdomen
- Check coat condition and musculature
- Weigh
- Obtain faecal samples and check for parasites immediately

Dehydration

- Sunken gaunt looking face
- Skin tenting over the back
- Sunken eyes
- Inactive and slow to move
- Often associated with emaciation
- Mouth very dry
- Tongue shrivelled

Breathing Assessment

- Coughing
- Breathing quickly than normal
- Noisy breathing
- Nasal discharge

What to do?? – Refer to sections 3.7, 3.8 and 3.14

- If active and mobile and no obvious signs of ill health
  - Offer oral rehydration fluids
  - Over the next 24 hours add some milk formula
  - By 48 hours can be on 80% strength formula
  - If OK then give full strength milk formula by 72 hours
  - Check skin for injuries and clean if necessary
  - Deworm and check skin for lice, ringworm

- If diarrhoea and dehydration mild
  - Offer oral rehydration fluids
  - Consider intravenous fluids but will need to be sedated
  - Check faeces - culture, wet prep and flotation
  - Treat with wormers, antibiotics and antiprotozoals - Metronidazole or tinidazole, Albendazole, Ciprofloxacin
  - Keep warm, relaxed, secure

- If severe emaciation, weakness associated with dehydration offer only small amounts of oral rehydration fluids for two to three days until starts to show some activity
- Do not give IV fluids even though there maybe severe dehydration – IV fluids may kill because the animal is not able to adapt to the rapid increase in sodium levels in fluids – often a severe infection can occur and cerebral oedema
- Treat any underlying problems but ensure given broad spectrum antibiotics
- Keep warm, relaxed, and secure

MEDICAL COMPLICATIONS & PROCEDURES

90% of all confiscated individuals are malnourished & sick. They are often emaciated, anorexic, hypothermic and infections are common.

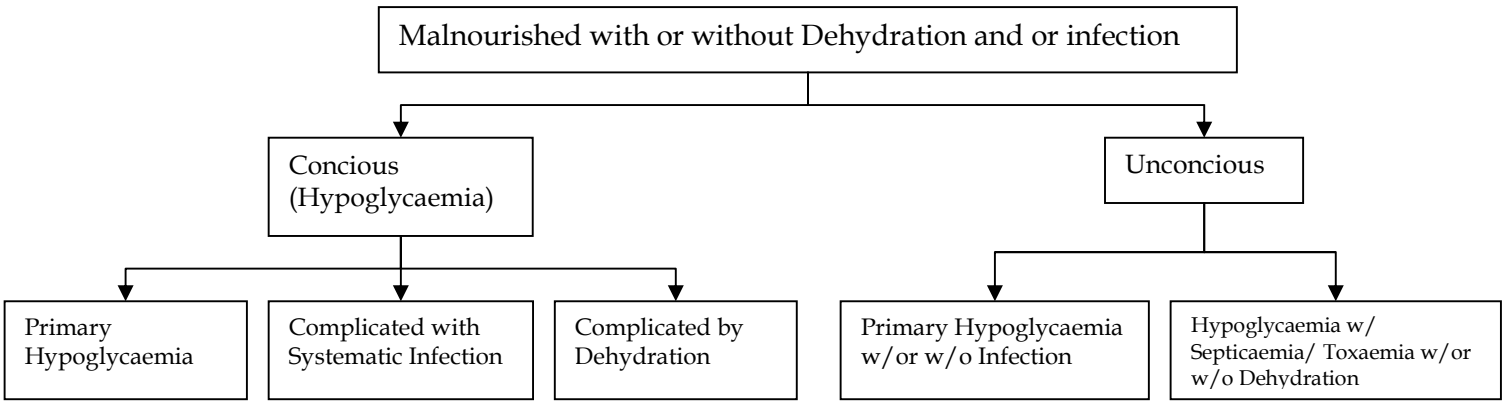


Figure 1 Complications of malnutrition (See also section 3.7)

MANAGEMENT OF CONFISCATED INDIVIDUALS

- Tasks of initial treatment are
  - Treat/prevent hypoglycemia
  - Treat/prevent dehydration & electrolyte imbalances
  - Treat incipient or developed septic shock
  - Start to feed
  - Treat infection
  - Identify & treat; anaemia, vit. def., cardiac

HYPOGLYCAEMIA (refer to 3.7)

- May be primary (> 4 hours starvation) or secondary to a heavy systemic infection.
- < 54mg/dl or < 3 mmol/l is definitive
- Other signs include: Low body temperature, lethargy, limpness and loss of consciousness
- Note: If suspected treat immediately w/o laboratory confirmation
  - Conscious individual - Give 50ml of 10% Glucose PO/ 1 water:4 50% Glucose
  - Unconscious/convalescent individual 5ml/kg body weight of sterile 10% glucose IV followed by 50ml of 10% Glucose by NG tube. Start with NG tube, proceed with 60g/l PO
- **All hypoglycemic individuals should be given Broad Spectrum Antibiotics**

DEHYDRATION (refer to 3.14)

Diagnose dehydration whenever you observe: history of diarrhoea, thirst, hypothermia, sunken eyes, weak/abscent radial pulse reduced urine flow & cold extremities. Other signs include: mental state, skin elasticity
NB: Always elect for oral rehydration whenever possible because IV fluids easily cause over hydration & heart failure. As such should only be considered when there are definite signs of shock. OG should always be the first choice over NG tube unless: there is vomition, weakness/exhaustion, fast breathing or painful stomatitis.

RATIONALE OF DEHYDRATION IN MALNOURISHED INDIVIDUALS

Because malnourished individuals are deficient in K+ and abnormally high Na+ the Oral Rehydration Salts (ORS) should contain less sodium and more potassium. The deficiencies of Mg2+, Cu2+ & Zn2+ should also be corrected

Composition: Standard ORS + 2liters H2O + 50mg sucrose + 40mls of mineral mix

MANAGEMENT OF ORAL DEHYDRATION (refer to 3.14)

- 70-100mls of ORS/kg body weight over 12hrs is usually enough to restore normal hydration

- Start with 5ml/kg every 30 min for 2hrs orally/NG tube and 5-10ml/kg per hour
- Reassess the individual per hour and stop ORS when resp> & pulse rates increase, jugular engorges & increased edema (puffy eyelids)

INTRAVENOUS REHYDRATION

- Circulatory collapse due to severe dehydration and septic shock is the only indicator for IV rehydration in a malnourished individual.
- Fluids of preference are in order of: Half strength Darrows w/ 5% Glucose, LRS w/ 5% Glucose & 0.45%(half normal) saline w/ 5% Glucose

MANAGEMENT OF IV FLUIDS

- Insert NG tube and administer ORS 10ml/hr as you set up the IV drip. Administer 15ml/kg IV over 1hour and monitor signs of overhydration.
- After 1 hour, respiration and pulse rates should fall, if not, repeat the procedure for another hour. If the individual gets better, maintain NG tube(10ml/kg/hour) for 10 hours otherwise treat for SEPTIC SHOCK

SEPTIC SHOCK

It may be incipient or developed septic shock. The former are always anorexic and should be fed with NG tube while developed septic shock shows some or all the following;
Clinical Signs

- Signs of dehydration w/o watery diarrhea
- Hypothermia & hypoglycemia
- Oedema and signs of dehydration

MANAGEMENT OF SEPTIC SHOCK

- Follow IV rehydration protocol
- Continue with NG tube as soon as the consciousness and radial pulse is restored.
- If there is no improvement in 1 hour or signs of Congestive are observed, give plasma/ heamacoel (10ml slowly over 3hrs).
- If there are signs of liver failure (purpura, jaundice, enlarged tender liver etc), give a single dose of 1mg vitamin K1
- If signs of congestive heart failure (distended jugulars, increased respiration or respiratory distress) are observed, administer a diuretic & slow rate of transfusion.
- NB Never use steroids or epinephrine

TREATMENT OF BACTERIAL INFECTIONS

- Detection of disease in the malnourished individuals is quite hard as they do not respond to the disease the same as the healthy ones
- It is actually scientific to administer broad spectrum antibiotics routinely whenever you receive a malnourished individual

- No apparent signs of infection and w/ no complications
- Cotrimaxazole 30mg BID for 5 days PO
- Complications i.e. septic shock, hypoglycemia, skin & urinary infections etc
- Ampicillin 50mg/kg IM/IV every 6hrs for 2days followed by amoxicillin 15mg/kg every 8hrs for 5 days

STABILIZATION PHASE

- Follows the initial 2 days
- Anemia, vitamin deficiencies, antihelmetics can be corrected during this time
- USUALLY PART OF QUARANTINE

2.5 VETERINARY ASPECTS OF HAND RAISING PRIMATES

AIM

- To produce a socially adjusted healthy individual that can be easily integrated into a larger mixed sex group without major psychological stress or injury i.e. Well adjusted
- Social Adjustment
- Stabilisation
- Quarantine
- Integration

QUARANTINE

- All individuals should go into quarantine immediately with a range of care givers who are sensitive to the needs of the individual
- Immediate care maybe needed – discussed elsewhere
- Keep isolated from other sanctuary animals but if there is another one that has recently come into quarantine, they should be in quarantine together unless there are specific health concerns. This is for integration reasons and socialising

CONSIDERATIONS

- Need to mimic mother's protection and bond while at the same time allow for continuous contact leading to integration at the earliest opportunity. This means simulating the normal mothering behaviours of the species concerned
- E.g.
    - Nursing for the first 70 days approx 10% of time
    - Juveniles carried initially by cradling until 5-7 months
    - Carried on mothers back from then onwards
    - Play behaviour
    - Socialisation with other juveniles

STABILISATION

- The health needs of the individual should be met
- May need intensive care
- General examination
- Eyes, ears, chest if possible, abdomen, skin, muscle, injuries,
- Weight
- Stool collection
- If young and needs milk give diluted with oral rehydration salts initially.
- Normal dilution of milk that is used for human babies – SMA, Lactogen, Esbilac
- Human bottles and teats can be used or cups if more advanced

- Approx 20-25% of body weight per day wet weight
- Feed every 2-3 hours including through the night initially if young
- Reduce to feeding from early morning to late night and hope that the nights are undisturbed
- Introduce solids from 15 weeks for chimpanzees 0- use a variety of items
- If in poor condition then add protein supplement to the milk e.g. Cerelac
- If on solids then ensure that the individual eats a variety of food and does not gorge on one type of food
- Produce records on food eaten, weights, temperatures, treatments
- The welfare needs of the individual should be met
- Sometimes need to sleep with caregiver to establish a feeling of protection
- Feed on demand – sometimes need to throughout the night
- Several healthy caregivers are good for socialisation
- Break contact with one caregiver by feeding and leaving immediately
- Contact with healthy dogs is good for play behaviour
- During the day – stimulate activities and allow them to discover items – termite mounds, ropes, trees, water
- Waa bark when the individual comes in contact with snakes, water
- Good to communicate using normal animal vocalisations

HEALTH PROBLEMS

- Diarrhoea – Bacterial, Parasitic (Enterobius, Hookworms)
- Respiratory infections - Common cold > to bacterial involvement
- Candida infection – oral
- Lice
- Ringworm
- Cuts and bruises

INTEGRATION

- Should take place as soon as possible with other animals of the same age if possible
- Sometimes it is stressful for the animal to go through the integration process
- Allow the chimp to become familiar with the facility and chimps it will join first
- Allow tactile access
- Mix with individuals of the same size or animals thought to be caring to form alliances
- Consider mixing with adult females that show keen interest or are known to be great foster mums – this is important when considering adding the juveniles with males who may cause harm
- Considerable screaming will happen when first left with the group
- Occasionally injuries can occur and sometimes some juveniles may need to be separated for a period
- Relationships are a dynamic thing and can change rapidly
- Sometimes the juvenile may need to come out of the group if very stressed

2.6 ESCAPE PROCEDURES

It is essential to have a well-formulated plan outlining procedures to be undertaken should an individual escape.

Escape should be prevented and measures put in place to review structural and procedural tasks to eliminate such potential. This is especially so when housing adult unpredictable individuals. It is imperative that no general panic ensues following an escape (shouting, running etc) as this may further precipitate the situation. Instead, a coordinated approach involving well-rehearsed drills should result in capture and restraint with minimum excitement.

Communication between all involved staff on the condition of the individual (injured, aggressive, etc), the general situation (location, numbers and identification of the individuals involved where possible), and whether the animal is contained within the compound of the facility or has ranged further a field. If the individual is outside the facility, its proximity to villages or roads is also essential to be communicated.

The written standard operating procedure should be easily adapted for various situations and must involve all staff that may be present at all times. The plan should then be incorporated into practiced drills and then carried out under various conditions including controlled/known times and confused/spontaneous circumstances so that the procedures become well known.

Immobilization is the most common method of re-capture. Other methods to consider in young or calm individuals are; hand-catching, netting, enticement using food rewards. Only in situations where there is likelihood of severe damage being inflicted that cannot conceivably be prevented could the use of a gun could be considered.

An immobilization kit should always be readily accessible with all the contents checked for presence and working order. Persons proficient and adequately trained in using the equipment should be designated. We would recommend Zoletil (Telazol) be used at a dose of 4mg/kg.

The individual(s) should then be carefully observed to ensure evidence of sedation (initial effects) and precautions taken early to prevent injury to the escapee, especially if at height or in a potentially dangerous/damaging area. Blankets, catch nets, or mattresses can be used to reduce injury.

Once fully sedated, careful approach and reassessment of level of consciousness should be ascertained. An assessment of vital signs and physical examination for injuries should be conducted and therapeutic measures carried out as required. All stages of the procedure of immobilization should be timed and recorded including doses. Standard anaesthetic monitoring procedures should be followed, paying careful attention to capture induced hyperthermia.

The individual should then be allowed to recover in a dark, quiet environment and monitored closely.

Later, once all other enclosures have been checked for security and all individuals accounted for, the reason for the escape should be elucidated and preventative measures outlined and enforced.

2.7 TRANSPORTATION

It is best to sedate chimps prior to transportation. However, young chimps (less than 3 years) can be crated and transported without sedation.

Crates should be strong with a solid bottom to contain urine and faeces. It should be lockable and bedding should be provided. Use of a cargo net as a secondary safety precaution is advisable. An experienced person (experienced vet if possible) should accompany the chimp during transportation. Emergency anaesthesia/ medical kit should be carried as well as a water spray/ ice cubes in case of hyperthermia. A suitable dose of an appropriate anaesthetic drug should be drawn up and ready for use.

2.8 STAFF HEALTH PROGRAMME

All efforts must be made to minimise the risk of disease being spread from humans to the primates or indeed vice-versa.

Before allowing staff to work with primates, staff should be tested for

- TB (skin test and radiology if possible)
- hepatitis A, B and C and
- ideally HIV infection
- And vaccinated against
- Polio, tetanus, hepatitis B.

All staff should undergo tests every six months for tuberculosis (intradermal test), hepatitis B and ideally HIV throughout their period of employment . Annual chest X-rays are also desirable.

In an endemic area many people will test positive on the intradermal skin test for TB.
If a staff member who has tested positive exhibits coughing or weight loss at any time, that staff person should be removed from contact with primates and further diagnostics performed.
If a staff member tests positive to TB and HIV, then he/she should not be allowed contact with primates.

NB: HIV+ve people are far more likely to contract TB than HIV-ve people, and hence are at greater risk of becoming carriers or spreading the disease.

All members of staff must agree upon these issues

All staff should undergo 6 monthly faecal testing for gastrointestinal parasites and bacterial pathogens. All parasite burdens and pathogenic bacteria should be treated. The first faecal test should be conducted before new staff come into contact with primates. Where this is not possible, staff should be wormed every 3 months.

New members of staff should not have any contact with primates for the first two weeks of employment. This should allow sufficient time for the development of most infectious diseases should staff be incubating any at the time of appointment. The two weeks should also allow sufficient time for faecal and blood testing and vaccination where appropriate.

Any member of staff suffering from respiratory symptoms or diarrhoea should not work with the primates until fully recovered. Consider some may shed chronically as a carrier status. Staff suffering from infectious skin diseases such as scabies should also be isolated from primates.

Standards of personal hygiene must be very high amongst primates' staff, although this may be easier to state than implement! Spitting, urinating or defaecating in the compound other than in the toilets must be strictly forbidden. In order to reduce the possibility disease spread between primates and humans, when handling primates at close quarters (e.g. when anaesthetised) staff should wear disposable gloves and a simple facemask.

Staff should advise any incidence of disease in their family members. E.g. colds, flu, measles, chicken pox. Staff should stay away from primates until the end of the incubation period of the specific disease

Staff should wash hands properly with soap and water before and after preparation of food, entering enclosures, contact with primates and after going to the toilet. Staff should shower at least once daily. Separate feeding utensils and cleaning tools should be used for each enclosure
Footbaths should be used at the entrance to enclosures. Organic material cannot be disinfected so foot grates/steel brush can be used to remove material. All food and excrement waste should be removed daily from the enclosure. The material should be placed in a pit and burned regularly.

OCCUPATIONAL HEALTH PROGRAMME

- Outline a model programme that was developed for US institution
- Realize that not all components are feasible
- Will illustrate what can be done to protect human health and also prevent disease transmission to apes
- Focus on hazards particular to working with primates

Participants in the Programme

Individuals that have "substantial animal contact" should be enrolled. Includes people who are in direct care of the animals or their living quarters on a regular basis. Also people who have direct contact with the animals (dead or alive), viable tissues, body fluids, or wastes on a regular basis. Consider including employee's immediate family members. Enrollment should be voluntary. Should occur prior to exposure to animals and other hazards. If a person declines to participate he/she should be assigned to duties that do not include contact with animals

History and Physical Examination

Medical history and pre-employment physical examination should be performed. Thorough history should be taken by trained health professionals. Chest X ray, CBC, chemistry panel, urinalysis, fecal examination for parasites, serum stored, tuberculin testing can also be performed.
Necessary immunizations can be initiated at this time. Annual physicals (and appropriate tests) should be performed. Physical examination after a hazardous situation such as a bite wound so the patient's health can be monitored.

Serum Storage

5ml of serum can be obtained and stored in two equal aliquots at -20 C. Taken upon enrollment, during disease outbreaks, and upon termination. Allows for retrospective analysis of exposure to infectious agents. Expensive and may not be economically feasible.

TB Testing

All individuals should have negative tuberculin skin tests prior to animal contact, or demonstrate that they are noninfectious. 0.1ml PPD tuberculin intradermally. Positive if 10 mm or greater induration at 48 hr. Chest X-rays and/or sputum culture for people who have positive reaction.
Some people will react due to prior BCG administration. Reactors should have a chest X-ray at time of reaction, annually thereafter and if clinically indicated. Converters prohibited from contact until they complete appropriate treatment.

Immunisations

This is the first line of defense for personnel potentially exposed to infectious agents. Vaccination status should be obtained upon employment. Vaccines provided if available.
Recommended vaccinations:

- Diphtheria, Pertussis, Tetanus (DPT): Childhood series and booster every 10 yrs
- Rubeola (Measles) and Rubella (MMR): Childhood series, boost after 18 yrs
- Polio: Childhood series, boost after 18 yrs with inactivated vaccine
- Rabies in endemic areas
- Hepatitis A and B: Initial series and booster every 5-10 yrs
- Yellow Fever: One dose every 10 yrs
- Others to consider:
    - Typhoid
    - Meningococcal meningitis
    - Influenza based on local outbreaks

Vaccines will need to stored appropriately. Consult local physicians

Records

- Centralized (computer?) record system
- Usually the responsibility of the health facility providing the service
- Duplicate records should be permanently maintained
- Historically has been poor record keeping
- Record all pertinent information including work assignments, exposure to hazardous agents, injuries, bite wounds, unusual illnesses
- Confidential information, therefore keep it in a locked cabinet
- Informed consent may be necessary

Education Programme

- Vital component
- Conduct regular training sessions
- Build trust between employer and employee
- Staff should understand the need for an occupational health programme
- Will increase the understanding of the risks
- Will promote behaviours that minimize risks
- Will increase knowledge about zoonoses

Health Services/Personnel

- Services available will vary from fully staffed hospital, or local clinic, or single physician to nothing! Assess what is available to you
- Brief the human doctors about the hazards of working with primates and educate them about zoonoses
- Establish effective channels of communication with doctors
- One person on staff should be responsible for overseeing the programme
- Consider what is feasible for you to provide from the very basic such as first aid treatment to a full programme
- Review the programme on a regular basis to ensure it is meeting your needs

Other Hazards

- Bites and scratches
- Knife and scalpel cuts
- Back injury, slippery floors
- Dangerous equipment
- Heat exhaustion
- Animal Allergies
- Chemical Hazards
    - Anaesthetics
    - Biological agents
    - Antimicrobials and other drugs
    - Disinfectants
    - Insecticides, herbicides
    - Formalin and other fixatives
    - Many others……..including Ionizing Radation especially with pregnant women

PRIMATE ZOONOSES

Who is most at risk?

- Children and the elderly
- Pregnant women
- People with chronic diseases
- Renal, hepatic, heart disease
- People who are immunosuppressed

- Diabetes mellitus
- Neoplasia, and associated treatments
- Splenectomy
- Malnutrition
- Chronic diseases (liver cirrhosis, etc)
- Hemodialysis
- HIV infection

Consult your physician if you are at higher risk before working with primates

COMMON ZOONOSES

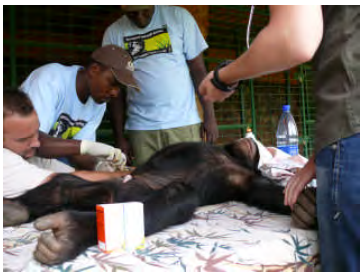
- Tuberculosis (see section 3.2)
- Shigellosis
- Salmonellosis
- Infectious hepatitis (A, B and C)
- Measles
- Rabies?
- Retroviral diseases (chimpanzees can carry HIV)
- Strongyloidiasis
- Entamebiasis
- Enterobiasis
- Balantidiasis
- Giardiasis
- Dermatomycosis

2.9 STAFF EMERGENCIES

A first aid kit should be available for emergency treatment of injuries in staff. The first aid kit must be routinely checked and any missing drugs/bandage material replaced immediately when missing or older than their expiry date. Staff should be trained on how to provide first aid. Ideally, an emergency procedure plan should be set up with a nearby hospital.
In case of an emergency the hospital should be notified immediately and the injured person transported there as fast as possible after/during the application of first aid.

SECTION 3

VETERINARY PROTOCOLS & PROCEDURES



SECTION 3 VETERINARY PROTOCOLS & PROCEDURES
Explaining the components of a preventative health programme

3.1 DISEASE RISK ANALYSIS

Steve Unwin and Dominic Travis

Additional material from the following sources:

- Geisecke J (2002). Modern Infectious Disease Epidemiology (2nd Ed) Arnold Publishers
- Sanderson S and Unwin S (2005). Chester Zoo contingency plan for HPAI. NEZS internal report.
- Thursfield M (2007). Veterinary Epidemiology (3rd Ed) Blackwell Publishing
- Travis D (2005) Veterinary Risk Analysis Lecture notes. PASA 2005 veterinary workshop

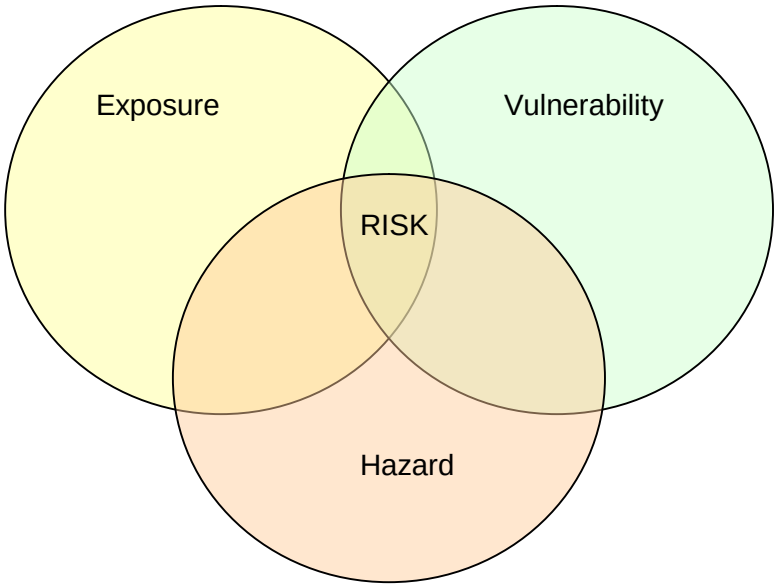


Figure 1. Risk of disease occurs where you have a hazard, (we will mainly be talking about pathogens here, but this could equally be something non infectious, such as too much sugar in the diet), a vulnerable population, who are then exposed to that hazard.

3.1.1 What Is Risk?

Risk is the likelihood of the occurrence and the magnitude of the consequences (severity) of an adverse event – for this you need a vulnerable population and the possibility of exposure, to a particular hazard (Figure 1). That is, risk is a measure of the probability (likelihood) of harm and the severity of the impact of a hazard. In veterinary risk analyses, hazards are usually a pathogen (e.g. virus) or a clinical sign (e.g. pneumonia). Objective measurement and scientific repeatability are key features of risk evaluation. In risk studies it is common, especially when discussing the risk orally, to use the term 'risk' synonymously with the likelihood (probability or frequency) of the occurrence of a hazardous event. In such instances the magnitude (severity) of the event is assumed to be significant.

3.1.2 Risk Analysis and Uncertainty

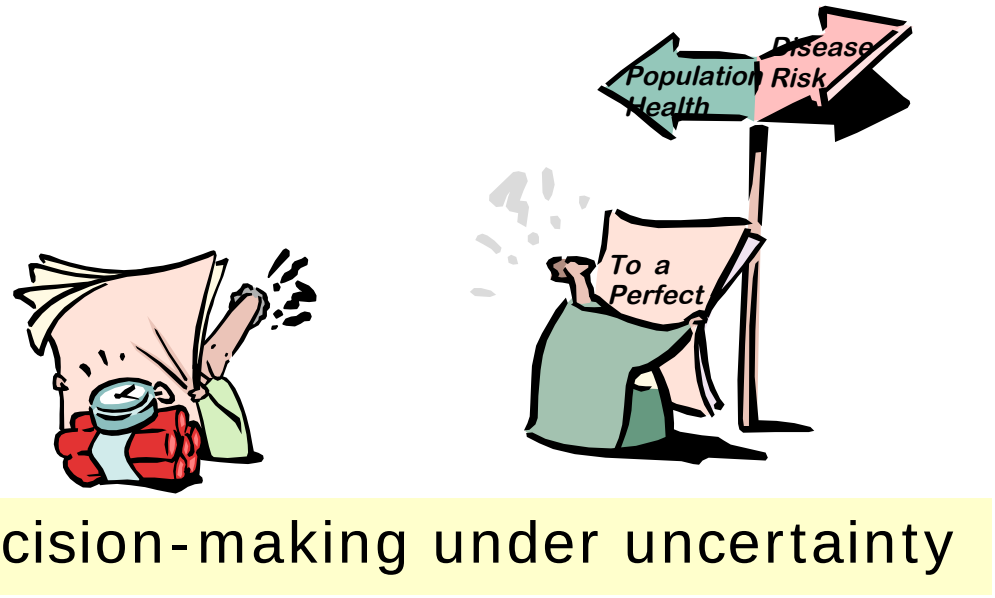


Figure 2. Risk Analysis assists decision making under uncertainty. It helps you analyse the 'ticking bomb' disease, at the crossroads of disease risk and population health.

There is often a large degree of uncertainty in deciding what is going to be a problem disease for your animals, and what may not be. Often information on disease risk and population health is scanty at best. By working through a risk analysis process, the aim is not only to highlight what we do know, or strongly suspect, but also where we need to focus our research efforts, to find out what we don't know. Risk analysis is a formal procedure for estimating the likelihood and consequences of adverse effects occurring in a specific population, taking into consideration exposure to potential hazards and the nature of their effects. This includes the management (usually reduction) of the likelihood of exposure.

Risk analyses are used widely in finance (e.g. to control either an individual's or companies chance of losing on an investment), in environmental science (e.g. to estimate hazards associated with contaminants or other environmental conditions, as they affect exposed humans, animals, or selected elements of an ecosystem), and in engineering (e.g. to study the safety of nuclear reactors).

Risk analysis is now applied to a wide range of veterinary issues. A major area is import risk analysis (reducing the risk of importing disease into an area). Examples include:

- Importation of Foot and Mouth Disease into the UK and South Africa (www.defra.gov.**uk**/animalh/**disease**s/monitoring/pdf/fmd_rsa.pdf)
- Importation of rabies into New Zealand (http://www.biosecurity.govt.nz/regs/imports/ihs/risk)
- Transmission of bovine tuberculosis from badgers to cattle in the UK (www.defra.gov.uk)
- Public health and animal health risks associated with Highly Pathogenic Avian Influenza around the world (Protocols in bundled notes on the workshop DVD under Avian Influenza)

This process has huge scientific merit when dealing with wildlife disease, as it provides a framework that can be followed when investigating disease.

3.1.3 Zero Risk in Veterinary Medicine?

Figure 3. A risky situation

No situation, in veterinary medicine especially, is without risk. Thus it is untenable to adopt a 'zero risk' approach. For example, it is unrealistic to aim for the absence of parasites in the primates in your sanctuary. So effective control of parasites must be tackled by identification of those animals that pose a risk, and management of the risk at critical stages through the year (or the parasites life cycle), and perhaps concentrating on biosecurity protocols to reduce spread of parasites.

Figure 4. Overeating macaques

Although we will be dealing primarily with infectious disease in this discussion, don't forget non infectious disease risk (as part of your preventative health plan). For example, malnutrition from either too little food, or the wrong type of food, or too much food and getting like the guys in figure 4.

Animal-health risk analysis has evolved to assess as objectively as possible the risks associated with particular diseases, rather than only relying on the somewhat subjective judgements of individual scientists or parties. All aspects of international animal disease control policy are now based on risk analysis, which has therefore become a routine veterinary procedure.

Veterinary risk analyses frequently report risk in terms of likelihood, but exclude severity from the assessment. This is often because severity may be very difficult to assess, due to the quality of the data available, or it is assumed that the severity will be high. For example, likelihood of diabetes in sanctuary gorillas may be negligible, but the likelihood of pneumonia in chimpanzees in the rainy season may be high. The severity of these two disease conditions may vary, but we often don't have the data to be able to quantify this, so we are more likely to investigate issues with pneumonia in chimps, because that is more likely than diabetes in sanctuary gorillas.

3.1.4 What Level Of Risk Is Acceptable?

This will vary with the assessment of each pathogen or group of pathogens. Thus, the results of risk assessments need to be meaningfully interpreted so that appropriate risk management strategies can be adopted.
We MUST be aware of the limitations of risk analysis which may pose more questions than it answers – indeed it is an important function of risk analysis to identify what is not known. The results of risk analyses may be founded on invalid assumptions, and frequently there may be uncertainty attached to the hazards and processes for which risk is being assessed. Moreover, because the severity of a potential risk is often difficult to quantify, it is often only possible to produce a relative ranking of the likelihood of the events, rather than accurate assessments.
Nevertheless, risk analysis has a valuable role to play in identifying and managing risk in many areas of veterinary medicine, rather than attempting to pursue a 'zero-risk' strategy, which would not be amenable to changes in the light of new knowledge, economic circumstances, or political requirements.

3.1.5 Risk Analysis Basics

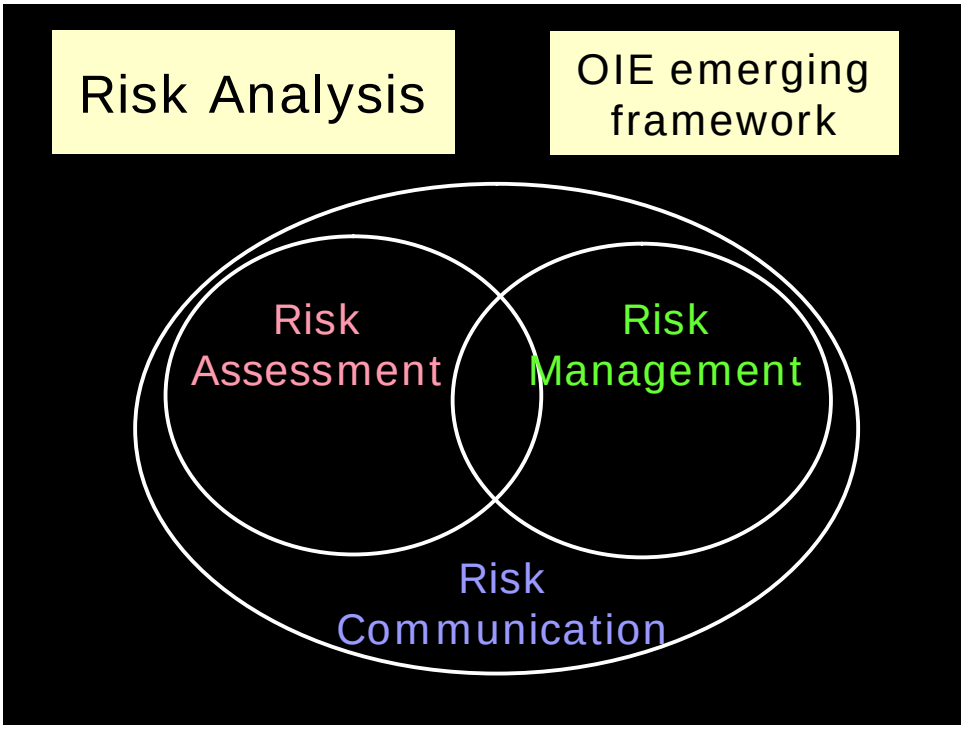


Figure 5. In its most basic form, a risk analysis consists of assessment to base management on, all of which is communicated to relevant parties effectively. This structure follows the OIE model of how the components of the risk analysis relate to each other. For more information on this, visit the OIE website www.oie.int

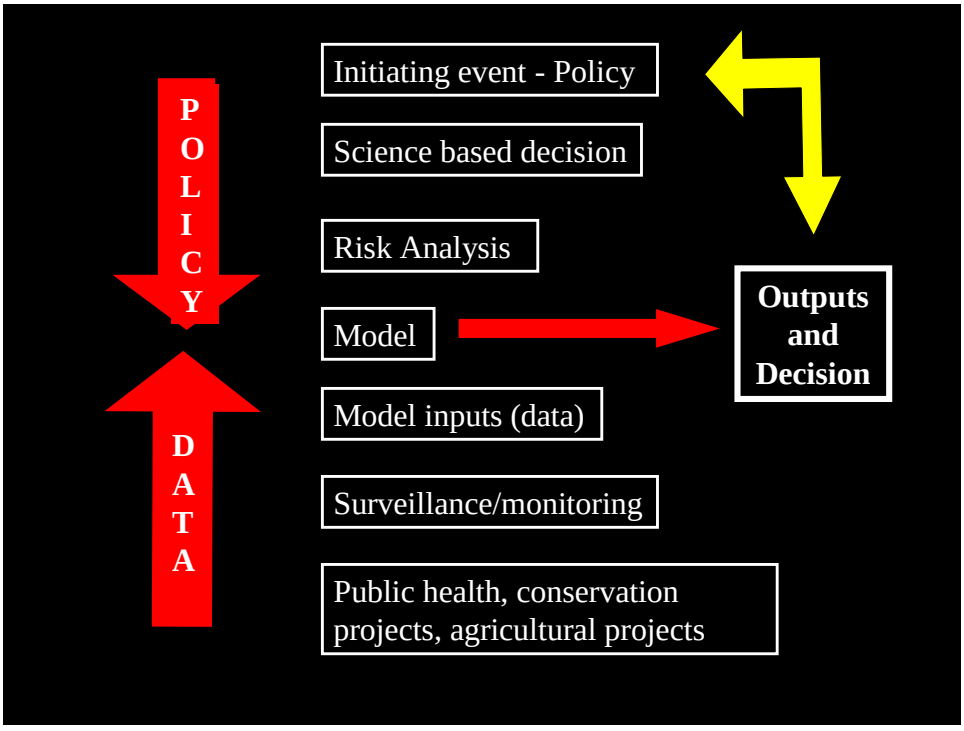


Figure 6. Feedback model to help inform policy decisions

Figure 6 illustrates a basic feedback model to help inform policy decisions. For example, the policy may be 'your sanctuary is proposing to minimise the risk

of diseases of concern in release chimpanzees'. By working through this model you can help pinpoint areas to help that policy become successful. The same would apply if you were updating the biosecurity protocols for your sanctuary, where the policy may be 'your sanctuary minimises parasite burdens to reduce the risk of clinical disease'. Thus, you want what you do to be scientifically based, for it to be best effective 'in the real world'. A risk analysis provides a framework to work through a disease issue scientifically. However, for it to inform a model that allows accurate and useful decision making, data is required. For veterinary disease investigations this data comes from surveying and monitoring your animals for your diseases and health issues of concern. This information can also be enhanced from local public health, conservation and agricultural projects.

WHAT ARE SOME OF THE POLICIES/ VETERINARY ISSUES AT YOUR SANCTUARY THAT MAY BENEFIT FROM THIS PROCESS?

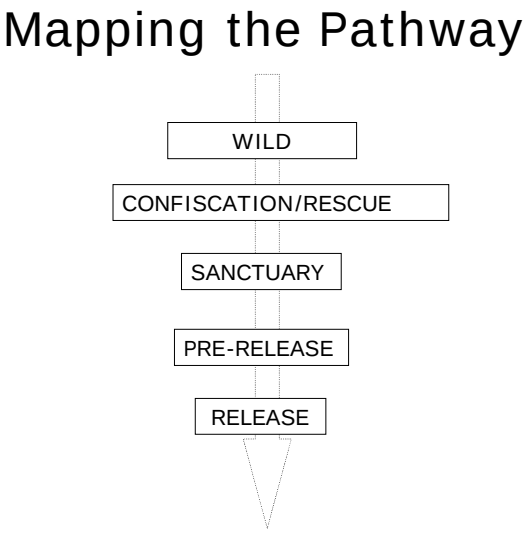


Figure 7. Mapping the pathway of a primate from the wild, to a sanctuary, and back into the wild via a release programme

As a first step – mapping the pathway of the policy you are trying to perform is essential. In the basic example of figure 7, a chimpanzee going from the wild, through rehabilitation, to release could be said to follow a pathway to release. By going through this process, you can remain focused on what areas may need monitoring for disease. You can also highlight where diseases (hazards) might crop up and cause a problem for your policy, but more on that later. PASA examples of mapping the pathway are shown at the end of this section (3.1.11)

3.1.6 Data Input – Qualitative Vs. Quantitative

The data that will be inputted into your model, and thus reduce the uncertainty of your analysis will be either qualitative or quantitative.
Qualitative data is information that is difficult to measure, count, or express in numerical terms. For example, data which measures intangible or objective data, such as peoples' attitudes and opinions, is qualitative.
Quantitative data is numeric information including quantities, percentages, and statistics.
When embarking on a disease investigation and risk analysis in non human primates, most of the data we have will be qualitative. However, as diseases are investigated more methodically (thanks to your good work!), the data gathered becomes more quantitative, thus reducing the uncertainty in any risk analysis we are performing. This also makes the risk analysis more precise – but does this also mean quantitative data will increase its accuracy? As sanctuary vets, you are on the cutting edge for obtaining this more quantitative data!

3.1.7 Risk Analysis – Asking The Right Questions.

Risk Assessment
- **What are the potential problems (hazards)?**
- **How important are they?**
- **When, where and how likely are they to occur?**

Risk Management
- **What can we do about them?**
- **How well will it work?**
- **How sure are we about our predictions?**

Risk Communication
- **Do the relevant people know about the risks and options?**

The questions above outline the basic components of a risk analysis. Figure 12 specifically relates these questions to a disease investigation scenario. Note that the data for the actual assessment may be either qualitative (high, medium or low) or quantitative (probabilities, percentages etc). It also highlights that management of diseases need not only be of a medical origin. Wildlife law and regulation also have a role to play, especially in managing exotic disease.

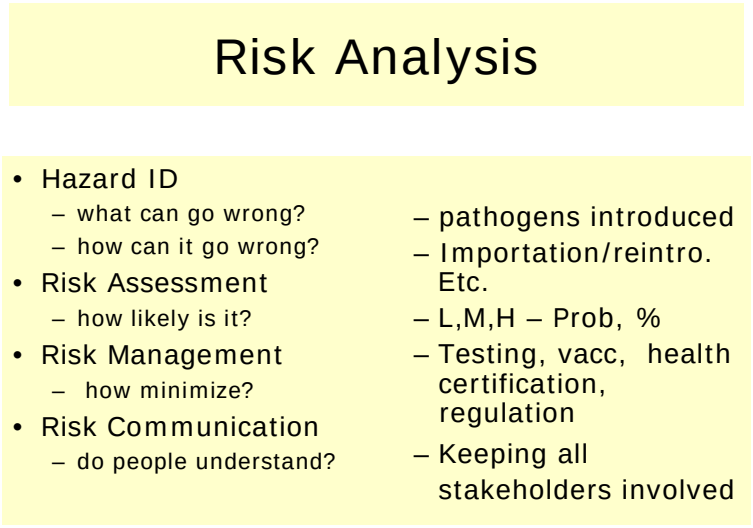


Figure 8. Combining the basic Risk Analysis questions with issues relevant to a disease investigation.

3.1.8 Hazard Identification and Risk Assessment

What follows are the steps involved in hazard identification and risk assessment.

Hazard Identification – To start the process you first need to know what hazards (pathogens) are relevant. This process is of course ongoing, as new information becomes available. Your hazard list will be based on published material, discussion with experts, sanctuary records, what is found in the local area etc

Hazard characterisation – Using your qualitative and quantitative data to characterise the hazard to the maximal level of precision known. For example, if the hazard is tuberculosis, can you say that the risk of TB in your sanctuary is low, or that the risk of importing TB into your sanctuary is high because the prevalence in the local area is 20%, or can you say 2 of the last 100 animals imported had confirmed TB, therefore the risk is moderate.

Release assessment – What happens when the pathogen gets out into the world? That is, what would be the consequences of you missing this hazard and it then being released in the wild? This where scenario trees, (a simple example is given in Figure 9), can come in useful.

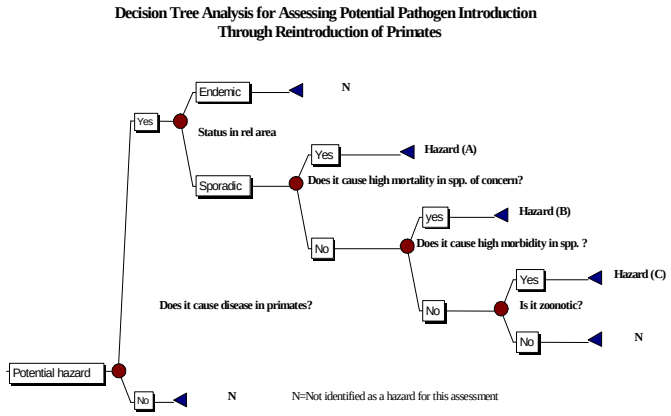


Figure 9. Decision Tree Analysis for Assessing Potential Pathogen Introduction Through Reintroduction of Primates

Exposure assessment – This means using whatever data you have to be able to assess the magnitude, frequency, duration and route of exposure of humans or animals to hazards. The assessment should also describe the size and nature of the population at risk. An assessment of the risk associated with the unrestricted importation of animals into the sanctuary for example, would consider the prevalence of pathogens in the source population, the probability of the pathogens surviving during importation, and the probability of the pathogens coming into contact with the animals and workers in the sanctuary. This information assists with mapping the pathway, because you can highlight points of entry for each disease of concern (see figure 15 and section 2.11).

Risk characterisation – This is the final part of the assessment and is a description of the nature and magnitude of risk, either to health or the environment. The description combines results of hazard identification, hazard characterisation, release assessment and exposure assessment.

Disease	Liklihood of susceptibility	Likelihood of exposure	Likelihood of becoming infected	Likelihood of transmitting to others	Severity to the individual if clinical	Severity for the population	Estimated significance to the programme
Ebola/marburg	5	1	5	3	5	5	24
Shigellosis	5	5	4	4	3	3	24
Salmonellosis (typed)	5	5	4	4	3	3	24
Strongyloidiasis	4	5	5	4	3	3	24
Hookworm	4	5	5	4	3	3	24
S.pneumonaie	4	3	4	4	4	4	23
Entamoeba hystolytica	4	5	4	4	3	3	23
Oesophagostomum	4	4	5	4	3	3	23
Campylobacter spp.	5	4	4	4	3	3	23
Tuberculosis	3	4	2	3	5	5	22
Enteropathogenic E.coli	5	3	4	4	3	3	22
Yersinia	4	4	4	4	3	3	22
Dermatophylosis	4	4	4	4	3	3	22
Balantidium coli	3	4	4	4	3	3	21
Anthrax	5	2	4	2	5	3	21
Rabies	4	3	3	3	5	3	21
EMCV	5	2	4	1	5	3	20
Giardia intestinalis	3	4	3	4	3	3	20
Cryptosporidia	4	3	3	3	3	3	19
Klebsiella spp	4	2	3	3	3	3	18
Malaria	3	5	3	2	3	2	18
Whipworm	4	3	3	3	3	2	18
Pinworm	4	3	3	3	2	2	17
RSV	3	3	2	3	3	3	17
Measles	3	1	3	3	3	3	16
Hepatitis B	2	4	2	2	3	3	16
Filaria	3	3	3	2	2	2	15
Sarcoptes spp.	2	3	2	3	3	2	15
SIV/ HIV	3	3	2	3	2	2	15
Polio	2	3	2	3	3	2	15
Pneumonyssus (mite)	3	2	3	2	3	2	15
Tetanus	4	2	2	1	4	1	14
Candidiasis	3	3	2	2	2	2	14
STLV	3	3	2	3	1	2	14
Amoebic meningioenceph	3	1	3	1	4	2	14
Hydatids/ Taenia	2	3	2	2	3	1	13
Hepatitis A	2	4	2	1	2	2	13
Influenza orthomyxovirus	2	2	3	2	2	2	13
Herpes simplex	2	4	3	2	1	1	13
Yellow Fever	1	2	2	2	3	3	13
Adenovirus	3	2	2	2	2	2	13
Pneumocystis carnii	3	2	2	2	3	1	13
Helicobacter	3	2	2	1	2	2	12
Parainfluenza III	1	2	2	3	2	2	12
Papilloma virus	3	3	2	2	1	1	12
Varicella virus	3	2	2	2	1	1	11
Cyclosporiasis	3	1	2	1	2	1	10
Hymenolepis nana	3	1	2	1	2	1	10
Hepatitis C	1	1	2	1	1	1	7

Figure 10. Primate diseases of Concern, categorised as high, medium, or minimal threats using a 'traffic light' colour system, based on the epidemiological assessment questions. The ranking of each category is from 1 (lowest significance) to 5 (highest significance) and is based on literature reviews and field data.

Many of you will be familiar with the list of diseases of concern in Figure 10, which is an example of combining hazard identification, hazard characterisation and exposure assessment to provide a risk characterisation by ranking, providing the sanctuary with a ranked 'Diseases of Concern' list.

WHAT ARE SOME OF THE DISEASES OF CONCERN FOR YOUR SANCTUARY?

WHY HAVE YOU CHOSEN THESE DISEASES?

WHAT IS THE EVIDENCE FOR THEM BEING A RISK?

3.1.8.1. Variability, Uncertainty and Subjectivity.

By going through this risk analysis process, 3 areas for error can be highlighted – variability, uncertainty and subjectivity.

Variability – is the inherent variation in biological systems (which can be managed by statistical manipulation). Variability will exist, even if there is complete knowledge of a situation. For example, it may be known that disease is present in the country, but the prevalence may be based on a sample survey, (a subset of the population), giving rise to a point estimate and a confidence interval for the prevalence of disease in the population as a whole. Thus while studies show a country may have a 20% prevalence for TB for instance, it is acknowledged that there will be a local variability of the risk of TB within a country or region. For example TB prevalence in Africa is assessed in this way.

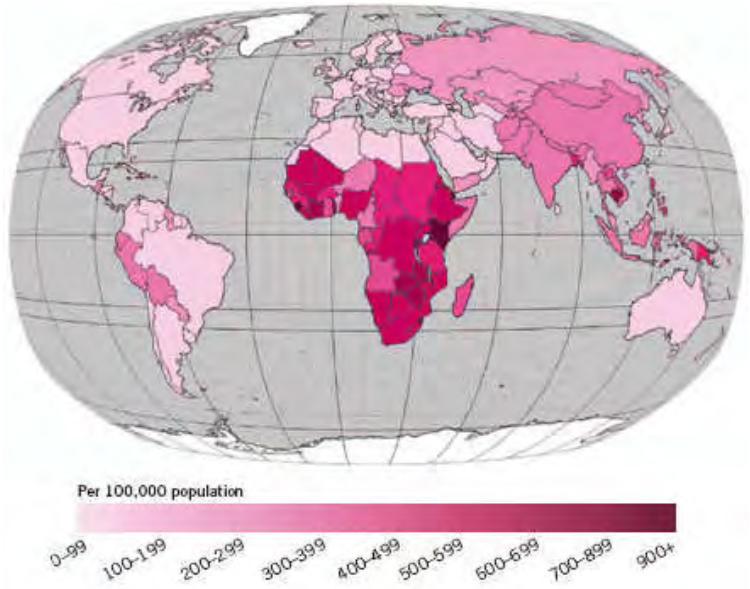


Figure 11. TB prevalence around the world – data based on surveys, rather than whole population censuses.

Uncertainty – this indicates ignorance. That is, a lack of knowledge of the disease status of a country. So this can be reduced with time as more data are collected and more research is conducted. For example – We are still uncertain as to the identification of the definitive hosts of ebola.

Figure 12: We are still uncertain as to the definitive host of ebola. Here are some potential candidates.

Subjectivity – Risk Analyses are always subjective, as the questions to be asked in risk analysis involve judgement, which is subjective. Therefore, to reduce subjectivity, all risk analyses should be fully documented and independently peer reviewed.

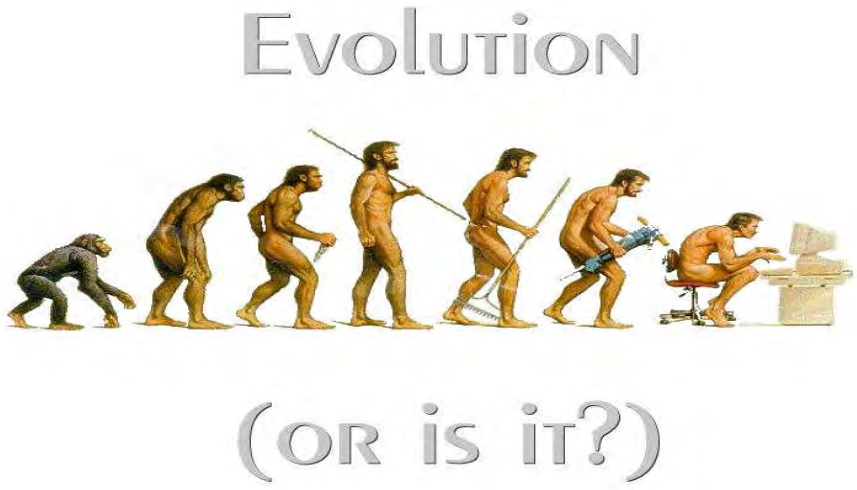


Figure 13. Deciding on who is most evolved will depend on your subjective point of view

3.1.8.2 Uncertainty Revisited

One of the major objectives of a risk analysis is to highlight areas of uncertainty

Table: Parasitology findings

Group	Adult (release group) (7-19yrs)	Petit (4.5-8.5yrs)	Quarantine (1.5-8yrs)	Nursery (1.5-2.5yrs)
N	15	10	12	4
Strongyloides culture positive	0%	40%	100%	100%
Balantidium coli	80%	30%	25%	0%
Hookworm	2/15 scanty			50%
Giardia	1/15		42%	
Entamoeba spp		20%		
Troglodytella spp.	0%	10%	75%	0%
Entamoeba coli	+	+	+	+
Fasciola spp		10%		

Negative for: Oesophagostomum, Whipworm, Pinworm, Dermatophylosis, Sarcoptes, Candidiasis, Taenia spp.
Not tested: Cryptosporidia, malaria. Malaria testing is being conducted at the end of 2007.

FINDING OF UNDETERMINED SIGNIFICANCE – change in risk analysis required. *Dientamoeba fragilis*, a potential pathogen thought to be associated with irritable bowel syndrome in humans, was found in one member of the petit group (not for release), and 1 staff member.

Figure 14. Slide highlighting parasitological findings from a survey conducted at the Centre for Chimpanzee Conservation

Figure 14 highlights some of the parasitological findings from the Centre for Chimpanzee Conservation in Guinea. It indicates that a protozoon of uncertain significance, *Dientamoeba fragilis* was found. It also highlights UNCERTAINTY in the significance of the protozoa. This parasite could pose a major threat to the CCC reintroduction plans (it doesn't!). This new potential hazard can be inserted into the model, and further data gathered on what is known about the parasite (hazard characterization). It can then be integrated into, or discarded from, the diseases of concern list.

DO YOU HAVE ANY HEALTH ISSUES IN YOUR SANCTUARY THAT YOU ARE UNCERTAIN REGARDING THE SIGNIFICANCE OF?

3.1.9 Risk Management

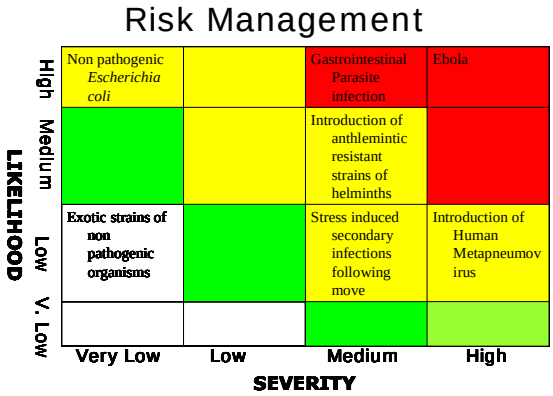


Figure 15. Risk Matrix for various primate diseases

Risk management can be prioritised by creating a risk matrix (Figure 15). For example, for the new Gorilla Rehabilitation Centre near the Tayna Nature Reserve in DRC, the likelihood of Ebola at the Centre might be considered medium or high, and the severity would also be high, based on what we know about the pathology of this disease. Therefore it is a disease of high concern. However, if this matrix was at Chester Zoo in the UK, although the severity for Ebola would still be very high, the likelihood would be very low (we don't currently import animals from ebola areas!). There is software available to assist in the development of risk matrices. For now, it is enough for you to know that risk matrices exist, and they may be a useful tool in risk management.

Limbe Wildlife Centre

Blood sample - minimum 10 mL - 6mL serum, 4mL EDTA, plus enough for 3 smears minimum, and several drops for filter paper. All samples to be duplicated
THIS IS A LIVING DOCUMENT AND WILL NEED TO BE UPDATED ON A REGULAR BASIS. THE SAMPLES HERE A A MINIMUM. ALL SANCTUARIES MUST HAVE ACCESS TO BLOOD EQUIPMENT AND FORMALIN AS A BARE MIN IMUM. TRAINING IN THE CORRECT USE OF THESE EQUIPMENT WILL ALSO BE REQUIRED FOR SEVERAL SANC

NOTES: On site veterinarian, in house laboratory. Note that this refers to the apes only. A second sheet for monkeys will need to be completed (SEE CERCOPAI

Disease Category	Aetiology (those in **bold** for inclusion in quarantine disease special interest)	Species	Relative Risk	Clinical Signs	Diagnostics	if blood samples, what tube, what volume?	for each samples, way and period of conservation	Who can test? (red current)	treatment if possible/ required	Husbandry	References/ comments	Test as part of normal protocol (T), test in face of outbreak (S)
Viral	**Hepatitis A,B,C**	All	L	Various - liver associated	Serology	serum (plain) and plasma (EDTA) 0.5mL	Freezing, months	JHI, Pasteur/ GAHMU			Not a disease issue, but may need to test for legal reasons?	T (Hep A and B only)
	Encephalomyocarditis virus	All	M	Sudden Death	Histopathology	N/A	Formalin, months	JHI/ GAHMU	N/A	Rodent control, cockroach control	More info??	S
	SIV/ HIV	Chimps	L	Usually assymptomatic	Serology	serum (plain) and plasma (EDTA) 0.5mL	Freezing, months	JHI, Pasteur/ GAHMU	N/A		Humans raise antibodies	T
	STLV	Chimps	L	Usually assymptomatic	Serology	serum (plain) and plasma (EDTA) 0.5mL	Freezing, months	JHI, Pasteur/ GAHMU	N/A			T
	Ebola/ Marburg	All	M	Sudden Death	Serology	serum (plain) and plasma (EDTA) 0.5mL	Freezing, months	CIRMF/ GAHMU	N/A			S
	Measles (morbillivirus)	All	L	Maculopapular exanthema	Clinical signs, virus isolation, seroconversion	serum (plain) and plasma (EDTA) 0.5mL	Freezing, months	JHI, Pasteur/ GAHMU	N/A		Vaccination?	S
	Polio (enterovirus)	All	L	Assymptomatic , CNS	Clinical signs	serum (plain) and plasma (EDTA) 0.5mL	Freezing, months	JHI, Pasteur/ GAHMU	N/A		Vaccination?	S

Figure 16 Part of a disease management chart – Limbe Wildlife Centre

Figure 16 shows part of a disease management chart, this one an example from Limbe Wildlife Centre. For a full list, please refer to Appendix 1. For each disease of concern, diagnostic methods and potential management strategies are given, both what is done, and what is ideal. Collation of this data is helpful so risk can be managed, in this case, across PASA, by highlighting, for example, what everyone considers important to test for, and highlighting potential laboratories to assist in investigating those pathogens.

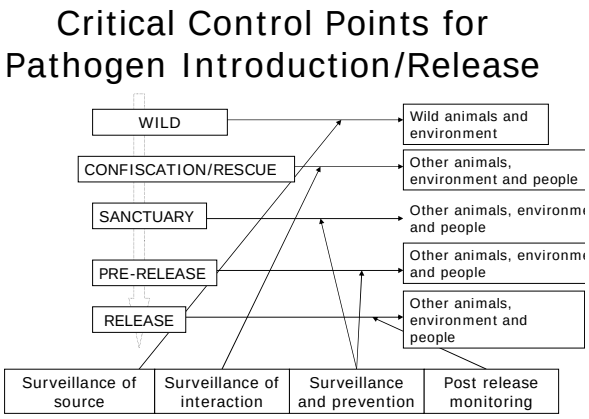


Figure 17. Critical Control Points on the Release Pathway

Returning to the Release Pathway, **critical control points** can be highlighted (Figure 17), to manage the risk of disease importation so resources can be utilized to best effect, (see section 3.1.11 for PASA examples). Remember however that the pathway isn't only about release situations. This sort of diagram is useful to draw up in any situation animals are moving, to allow you to highlight critical control points for disease management.

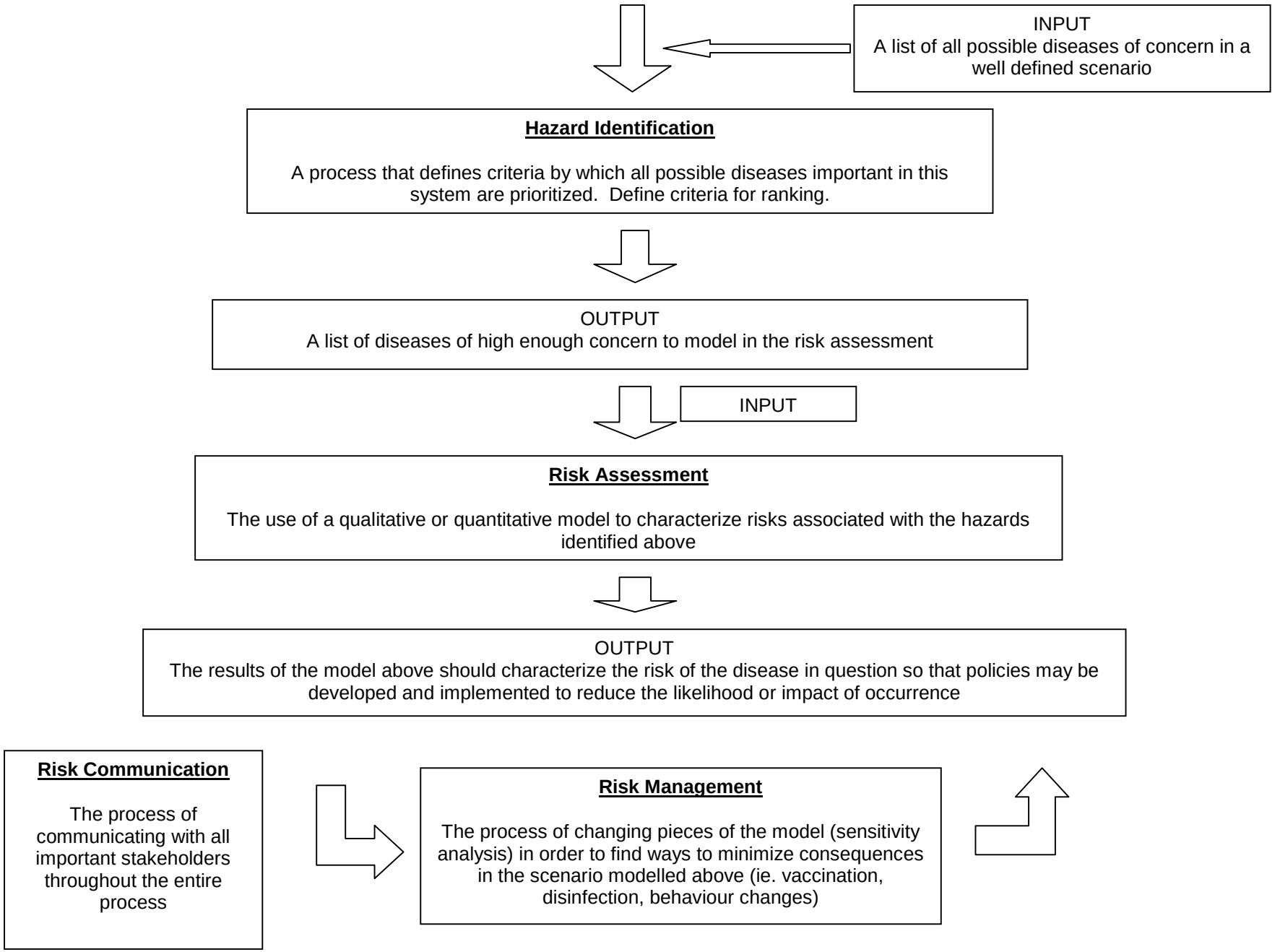
3.1.10 Risk Communication

The most important step in the risk analysis process is communication of the risk to all interested parties (your manager, your staff, other vets, your government, peer reviewed journals, BBC etc.), AND encouraging dialogue between them. Risk communication is particularly important because the perception of risk by people who do risk analyses is often very from that of the general public – such as the local village elders, or your manager (!). The former (us) may argue that risk should be determined objectively by the 'data alone', whereas the latter may 'irrationally' colour their perception of risk by subjective factors – often called 'outrage factors'. Reality is usually somewhere in the middle.
Since society generally reacts more to outrage than 'mere hazard', an important part of risk communication is to make serious hazards 'more outrageous', and modest hazards less so. Gruesome graphic government campaigns highlighting the dangers associated with driving under the influence of drinking or drugs, or some of the educational material used to inform on the transmission of ebola (Figure 18) are examples of increasing outrage. The extent to which the 'public' accepts risks is clearly related to the degree of outrage.

Figure 18. Image from a series of educational cartoons on the spread of ebola in the Republic of Congo (Thanks to Ken Cameron, WCS Field Vet Programme)

So, risk communication should not be an afterthought. Consideration of communication of the results of a risk assessment is essential in both defining the hazard and the risk question, as well as formulating the approach to the whole risk analysis. Otherwise the whole exercise will be rendered useless.

3.1.11 RISK ANALYSIS OVERVIEW



PASA VET CONFERENCE 2005

RISK ASSESSMENT

Group 1

Richard Ssuna (Ngamba Island, Uganda)
Sheri Speede (Sanaga-Yong, Cameroon)
Jason Boyer (Chester Zoo, UK / Limbe Wildlife Centre, Cameroon)
Felix Lankester (Limbe Wildlife Centre, Cameroon)
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Ann Pass (Cercopan, Nigeria)
Tafon Godwin Babila (Cameroon Wildlife Aid Fund)
Adeuiyi Egberade (Pandrillus, Nigeria)

Summary

At the moment there are 3 sanctuaries within Cameroon working with chimpanzees of the subspecies *troglodytes* and *vellarosus.* These are the Limbe Wildlife Centre, Sanaga-Yong Chimpanzee Rescue Centre and the Cameroon Wildlife Aid Fund. Our risk assessment covers a possible inter-sanctuary collaboration for a possible release site for the chimpanzees kept within these sanctuaries. The site will be subspecies specific and the risk assessment would work for both subspecies.

Questions

Probability of introducing chimpanzee x into the wild with disease y

1) Probability of chimpanzee x leaving critical control point 1 a, b and c with disease y.

a) Probability of chimpanzee x arriving at one of the sanctuaries with disease y.
b) Probability of chimpanzee x contracting disease y while in quarantine.
c) Probability of disease y going undetected while in quarantine

2) Probability of previous infection surviving transport or introduction of the agent during transport from critical control point 1 a, b and c.

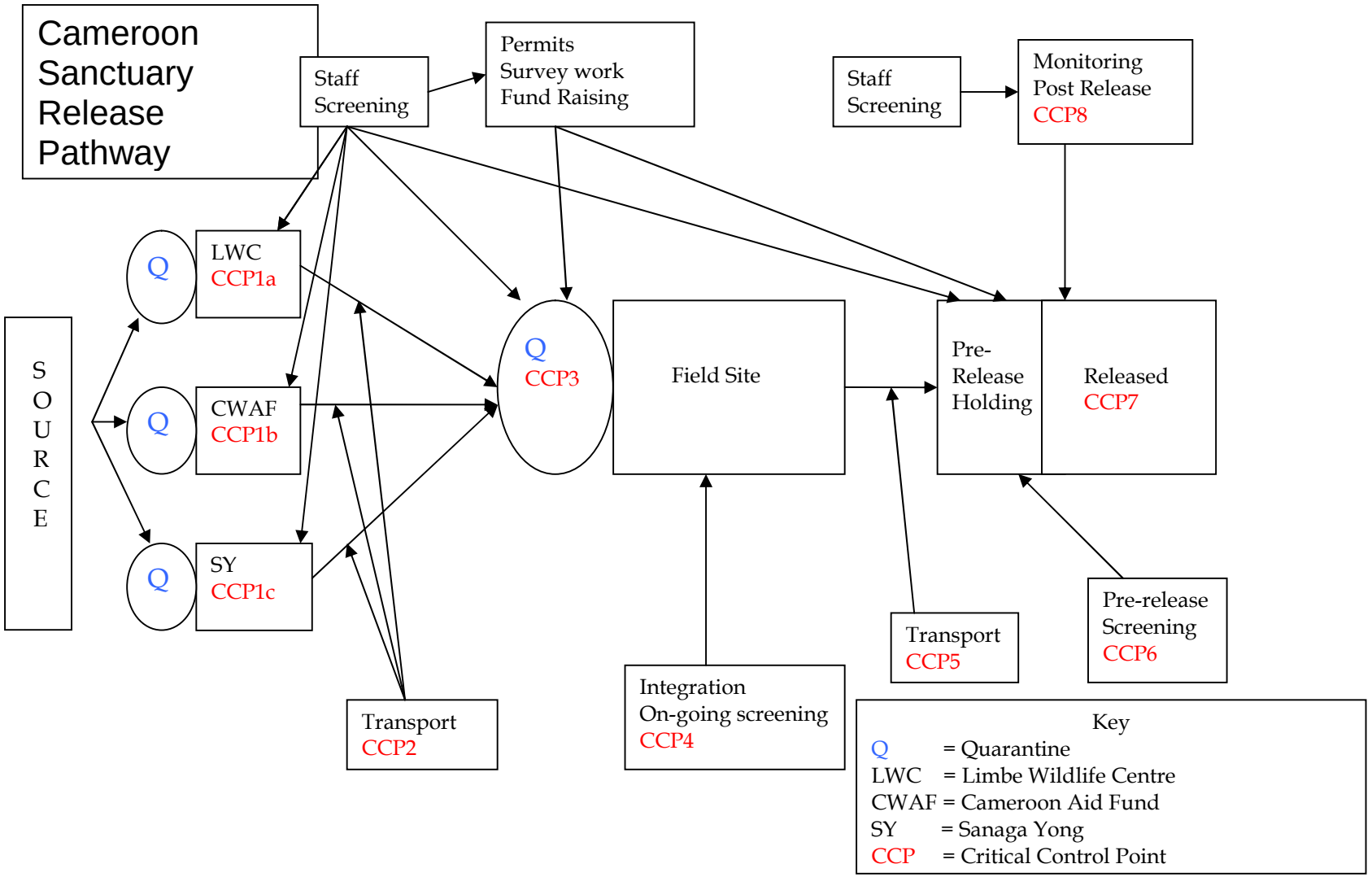
a) Probability of the agent survival.
b) Probability of the introduction of the agent during transport.
c) Probability of the hosts survival.

3) Probability of chimpanzee x leaving the field site with disease y.

a) Probability of a FN at critical control point 1.
b) Probability of a FN at critical control point 2.
c) Probability of an introduction of a new infection or a FN at the field site.

4) Probability of a previous infection surviving transport or an introduction of a new infection during transport from the field site to the release site.

a) Probability of the agent survival.
b) Probability of the introduction of the agent during transport.
c) Probability of the hosts survival.


Cameroon Sanctuary Release Pathway
Staff Screening
Permits
Survey work
Fund Raising
Staff Screening
Monitoring
Post Release
CCP8
SOURCE
Q
LWC
CCP1a
Q
CWAF
CCP1b
Q
SY
CCP1c
Q
CCP3
Field Site
Pre-Release Holding
Released
CCP7
Transport
CCP2
Integration
On-going screening
CCP4
Transport
CCP5
Pre-release
Screening
CCP6
Key
Q = Quarantine
LWC = Limbe Wildlife Centre
CWAF = Cameroon Aid Fund
SY = Sanaga Yong
CCP = Critical Control Point

Group 2.

Christelle Chamberlan (PPG Congo)
Eric Dubuis (PASA)
Tony King (PPG Congo)
Ken Cameron (Tchimpounga)
Carmen Vidal (HELP Congo)
Catherine Sourmail (HELP Congo)

Summary: Using Tchimpounga Sanctuary as a model, we examined the existing flow structure of animals passing through Tchimpounga in relation to a theoretical chimpanzee release program. In the diagram below, critical control points for disease are marked with a red number. We asked the primary question:

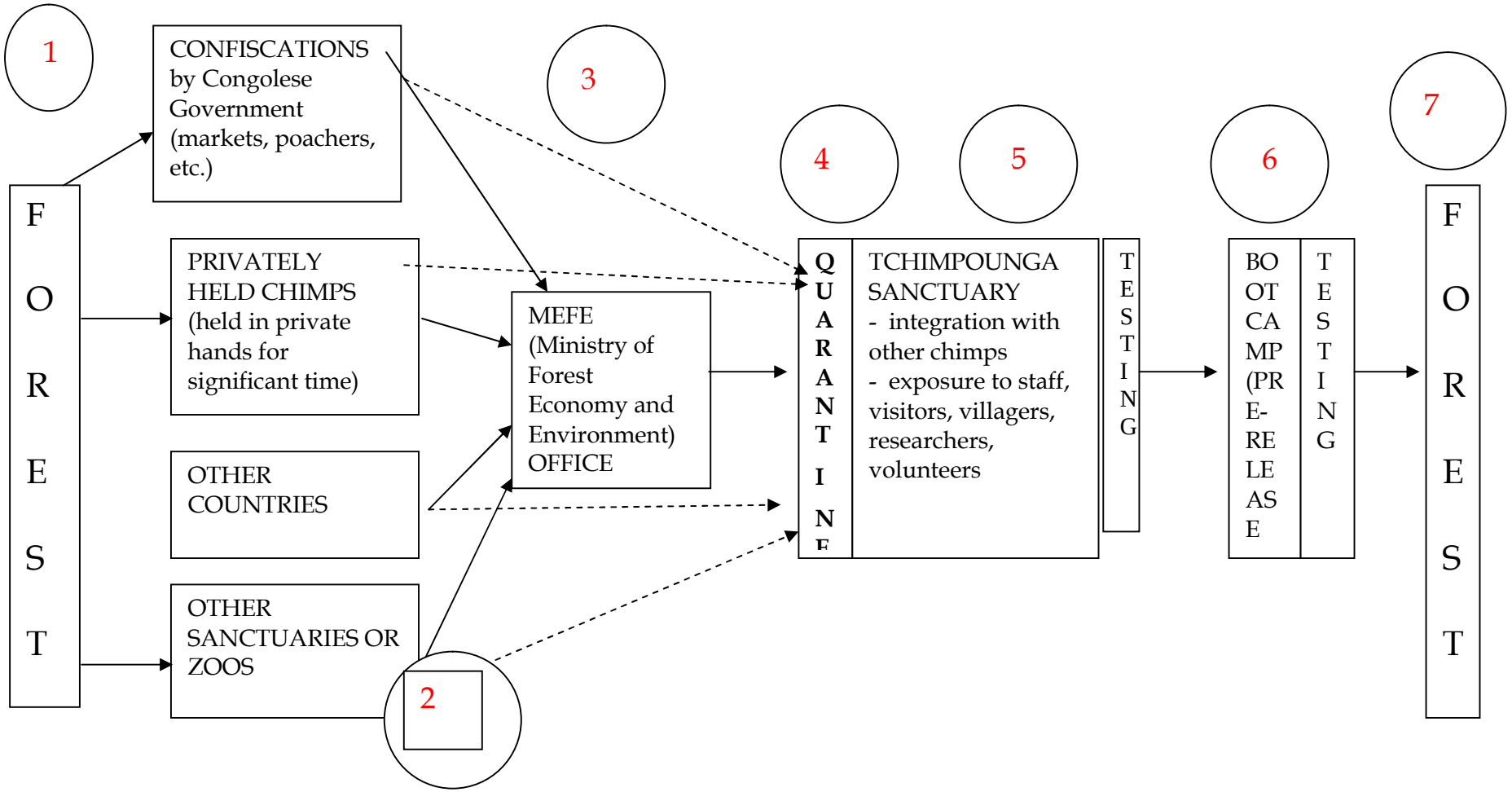
What is the chance that an infected animal will be released into the wild?

A. What is the probability that an infected animal will leave quarantine infected?
    1. What is the probability that an animal will be caught infected?
2. What is the probability that an animal will become infected in captivity prior to confiscation?
3. What is the probability that an animal will become infected at the MEFE (Ministry of Forest Economy and Environment) office?
    4. What is the probability that an animal will become infected in quarantine?
5. What is the probability that quarantine testing will miss something?

B. What is the probability that an infected animal will leave the sanctuary infected?
    1. What is the probability that an infected animal will leave quarantine infected?
    2. What is the probability that an animal will become infected in the sanctuary?
    3. What is the probability that testing prior to leaving the sanctuary will miss something?

C. What is the probability that an infected animal will leave the "Boot Camp" infected?
    1. What is the probability that an infected animal will leave the sanctuary infected?
    2. What is the probability that the animal becomes infected in the "Boot Camp"?
    3. What is the probability that testing prior to leaving "Boot Camp" will miss something?

D. What is the chance that an infected animal will be released into the wild?
    1. What is the probability that an infected animal will leave the "Boot Camp" infected?
    2. What is the probability that an animal will become infected during transport and release?


1
CONFISCATIONS by Congolese Government (markets, poachers, etc.)
3
4
5
6
7
FOREST
PRIVATELY HELD CHIMPS (held in private hands for significant time)
MEFE (Ministry of Forest Economy and Environment) OFFICE
OTHER COUNTRIES
OTHER SANCTUARIES OR ZOOS
2
QUARANTINE
TCHIMPOUNGA SANCTUARY
- integration with other chimps
- exposure to staff, visitors, villagers, researchers, volunteers
TESTING
BOOTCAMP (PRE-RELEASE
TESTING
FOREST

3.2 DISEASE CONTINGENCY PLANNING – AN EXAMPLE OF DISEASE RISK MANAGEMENT

Author (template) S.Sanderson. Authors (Content): Delegates of the PASA 2008 Vet Workshop

In disease investigations, contingency planning is mainly used for exotic disease. In the sanctuary situation, these plans should lay out procedures to prevent spread of a particular disease between the sanctuary and the wild, or a confiscation site and the sanctuary. We went through several exercises at last year's workshop to help you with starting to contingency plan for disease import. These are in the vet manual. Writing these plans down, in a format of most use to you, allows you to manage a situation clearly (everyone knows their role) and provides evidence to outsiders that you have been thinking about these issues. These plans indicate you have tried to the best of you ability and resources to prevent a major disease import or export. This point can not be emphasised enough especially as this sort of thing is becoming mandatory for PASA sanctuaries.

Disease of Concern: *TUBERCULOSIS*

Aim**:** to decrease likelihood to introduction of tuberculosis to or dissemination from a PASA sanctuary.

Principles: Control measures designed to reduce the risk of transmission.

Routes of possible transmission and contingencies undertaken are listed below.

Main Routes of transmission	**Contingencies to reduce risk of transmission to/from Sanctuary animals.**
Wildlife and Domestic animals	Aim: reduce contact between wild animals and sanctuary animals: • Domestic cattle around the sanctuary can be vectors. • Wildlife mammal vectors are likely and will vary between sanctuaries **Preventative measures:** • Prevent contact between primate's enclosures and domestic cattle, not allowing them to graze in the same area. • Minimise contact between wildlife mammals and primates as much as is practical
New Arrivals	Aim: Prevent introduction of infected animals. **Control measures:** • If possible, ask for certified diagnostic test before arrival. Obtain as much history on tuberculosis in all populations, from the area of origin, as is possible • Quarantine ➢ Different animal care staff from the sanctuary should administer quarantine. ➢ Length: 90 days to identify classical symptoms ➢ Intradermal skin test: Two tests to be undertaken during quarantine, 42 days apart, using Mammalian old tuberculin, avium and bovine tuberculin

	➢ Utilise serology rapid test if available ➢ Thoracic radiology, if possible. ➢ Sputum and tracheal lavage, if possible. Definitely take tracheal lavage for culture if other testing reveals a possible positive.
Food	Aim: Prevent entry of the disease in infected food products. Food items are not a common source of TB. **Control measures:** 1. Controlled origin of the food, specially the green that we often offer to our animals
Fomites (vehicles, equipment, crates, clothing and shoes etc.)	Aim: Prevent disease being transferred to animals, their food or anything they may come in direct contact with. **Control measures should disease be widespread (outbreak):** 1. Footwear disinfected to all of trucks and cars (wheels and wheels arches) that enter to the quarantine and the sanctuary area.
Faeces / waste food/ soiled bedding etc.	**Control measures in the event of outbreak:** **1.** Wasting products form suspected animals or enclosures must be packet and sealed carefully and separately form others products 2. Daily disinfection of soil with approved products for MT
Infected Humans	Prevention of transfer of a disease strain that can infect both humans and animals. • We would like to make a difference between working staff and visitors • Efforts should concentrate on keeping our staff healthy. **Recommendations** Visitors: • In case of outbreak restrict access to the centre. • Always wear facial masks when entering the centre • Small questionnaire about health status to be undertaken • Prevent visitor access if exhibiting respiratory symptoms. • Not less than 10-15 meters between animals and visitors Staff: • Prophylactic health programme: In vitro Quick test and Test de Mantoux. • Work wearing facial masks and gloves.

Additional points:

- These contingency measures are liable to revision as the threat changes and our knowledge of the disease and its control develops. They will be reviewed on a regular basis (minimum monthly).

- The contingency of how we would operate and provide care for our animals in the event of a human pandemic is also not covered within this document.

Summary table:

Measures in place (DATE):	• Test of intradermal reaction against M.tuberculosis and bovis • Quarantine
Measures to be put into effect ASAP: Timing to be supplied as soon as they are known.	Sanctuary Dependant Sanctuary dependant
Measures to be put in place if outbreak:	• Isolation the sanctuary and positive animals • Stop animal movements • Check all the collection with quick test and intradermal reaction (M. Tuberculosis, avium and bovis) • Information the authorities • Possible sacrifice of positive animals

2. Disease of Concern: *HMPV (Human Metapneumonia Virus)*.

Main Routes of transmission	**Contingencies to reduce risk of transmission to/from Sanctuary animals.**
Wildlife • humans • primates	Aim: reduce contact between humans/other primates and sanctuary animals: • Staff • Visitors • Free-ranging primates **Preventative measures:** • 1. Prevent staff with clinical signs of respiratory disease from working with sanctuary animals; wear masks; hygiene (e.g. hand washing); staff health programme (including immediate family if possible) including screen for HMPV. New staff/volunteers to work separately from animals for one week, monitoring for clinical signs of respiratory disease (i.e. same measures as for visitors). • 2. Minimum distance for visitors to sanctuary animals (at a 'safe' distance to reduce transmission of disease); prevent ill visitors (with respiratory disease) from entering sanctuary; wear masks.

	• 3. Biosecurity – fencing to prevent access to sanctuary by free-ranging primates.
New Arrivals	Aim: Identify infected animals (could be carriers). **Control measures:** • Quarantine – refer to PASA's minimum standards for quarantine. • While in quarantine, run test for disease (laryngopharyngeal dry swab for PCR, can be stored at room temperature). • Monitor for clinical signs of HMPV during quarantine & after mixing with other sanctuary animals. • Optimise husbandry of all sanctuary animals to reduce risk of other diseases that may predispose respiratory infection (e.g. bacterial pneumonias) that may result in severe disease in conjunction with HMPV.
Food	Aim: Reduce risk of entry of the disease in infected food products. **Control measures:** • Staff & visitors to wear facemask in food storage/preparation areas. • Humans not permitted in food areas if have signs of respiratory disease.
Fomites (vehicles, equipment, crates, clothing and shoes etc.)	Aim: Prevent disease being transferred to animals, their food or anything they may come in direct contact with. **Control measures should disease be widespread (outbreak):** • Instigate quarantine in enclosure containing outbreak: increase biosecurity & personal protective equipment (i.e. facemasks, foot baths/clothing change between enclosures). • Use separate cleaning equipment, food bowls etc in enclosure with outbreak (to reduce risk to animals in other enclosures). • If possible, separate staff to deal with animals in outbreak enclosure. If this is not possible, deal with this group after others in sanctuary.
Faeces / waste food/ soiled bedding etc.	**Control measures in the event of outbreak:** • Observe quarantine. • As for fomites: safe disposal of material from enclosure with outbreak.

Infected Humans	Prevention of transfer of a disease strain that can infect both humans and animals. • In the event of a human pandemic with a virus that is capable of causing serious disease in our animals, prevention is likely to be impossible. **Recommendations** Efforts should concentrate on keeping our staff healthy.

Additional points:

- These contingency measures are liable to revision as the threat changes and our knowledge of the disease and its control develops. They will be reviewed on a regular basis (minimum monthly).
- The contingency of how we would operate and provide care for our animals in the event of a human pandemic is also not covered within this document.

Summary:

Measures in place (DATE):	(Limbe Wildlife Sanctuary) • Staff health programme e.g. ill staff not to work with animals • Quarantine of new arrivals
Measures to be put into effect ASAP: Timing to be supplied as soon as they are known.	• Enforce guidelines regarding wearing of facemasks by staff & visitors; enforce strict sanitation. • Evaluate 'safe' distance between visitors & sanctuary animals, enforce this distance. • Include screening for HMPV in staff health programme & of new arrivals of animals. • Screen (quiz) visitors regarding respiratory disease.
Measures to be put in place if outbreak:	• Quarantine of affected enclosure(s). • Closely monitor animals & humans for signs of respiratory disease (enabling rapid treatment of animals with disease).

3. Disease of Concern: ***EBOLA***

Main Routes of transmission	**Contingencies to reduce risk of transmission to/from Sanctuary animals.**
Wildlife	Aim: reduce contact between wild animals and sanctuary animals: Define sensible species: Primates, antelopes, elephants, humans…

	Define potential carriers: fruit bats, rodents **Preventative measures:** • Prevent animals to enter the sanctuary: ➢ Fruit bats: light where bats stay (in facilities), close potential entrances in the buildings; stock food in a secure building ('bats and rodents proof') ➢ For primates and wildlife: buffer zone around the sanctuary and enclosures, double enclosures • Stop the walks in the forest with your primates • Health monitoring of wild populations by experts
New Arrivals	Aim: Prevent introduction of infected animals. **Control measures:** ➢ "Pre-quarantine", stabilisation of 2 weeks for any new arrival animal ➢ Quarantine of 3 months with really strict hygiene protocol for staff dedicated to the quarantine, equipment dedicated to the quarantine area (same for pre-quarantine), clothing, foot bath, masks… • Blood testing and any test required • Suspension of new arrivals
Food	Aim: Prevent entry of the disease in infected food products. **Control measures:** ➢ Storage in a really secure building, where no bats or rodents can enter • Disinfection of food prior to feeding
Fomites (vehicles, equipment, crates, clothing and shoes etc.)	Aim: Prevent disease being transferred to animals, their food or anything they may come in direct contact with. **Control measures should disease be widespread (outbreak):** • No public access to the sanctuary • No transfer of staff between different zones ➢ Equipments dedicated to each zone, disinfected as often as possible ➢ Keep one minimal team on site that do not go outside anymore • Lock the animals in indoor facilities
Faeces / waste food/ soiled bedding etc.	**Control measures in the event of outbreak:** ➢ All waste from enclosures will be bagged and sealed in dedicated plastic bags. These will be spread down with disinfectant on collection, and disposed to landfill as usual ➢ In case of suspect death: special equipment (high security) for people manipulating the body, necropsy to collect samples, and incineration of body and spread down with lime

Infected Humans	**Prevention of transfer of a disease strain that can infect both humans and animals.** • In the event of a human pandemic with a virus that is capable of causing serious disease in our animals, prevention is likely to be impossible. **Recommendations** • Efforts should concentrate on keeping our staff healthy. • No visits from outside • Isolation of people in the sanctuary, nobody goes outside (except people to get food for everybody, who get disinfected – as well as vehicles and equipment – when they enter the sanctuary) • Vaccination of people and animals if and when available

Additional points:

- These contingency measures are liable to revision as the threat changes and our knowledge of the disease and its control develops. They will be reviewed on a regular basis (minimum monthly).
- The contingency of how we would operate and provide care for our animals in the event of a human pandemic is also not covered within this document.

Summary:

Measures in place (DATE):	• Place new arrivals in isolation for 2 weeks with really high standard hygiene condition, then in quarantine • Store food in a really secure building and disinfect food before feeding
Measures to be put into effect ASAP: Timing to be supplied as soon as they are known.	• Prevent contact between wildlife and primates of your sanctuary (buffer zone, double enclosure…) • Prevent fruit bats and rodents from having any contact with the food for the primates
Measures to be put in place if outbreak:	• Nobody (humans and animals) enter the sanctuary • Minimal team of staff stays in the sanctuary all the time • Use foot bath, masks… • Only essential vehicles to enter the sanctuary (must be washed down and disinfected first) • Lock the animals in indoors facilities

4. Disease of Concern: Encephalomyocarditis Virus

Main Routes of transmission	**Contingencies to reduce risk of transmission to/from Sanctuary animals.**
Wildlife	Aim: reduce contact between wild animals and sanctuary animals: **Rodents mainly** **Potentially many mammals, birds and arthropods** **Preventative measures:** Hygiene, traps, pest control, removal of food of the feeding points, cleaning and routine disinfection, cats, vaccination
New Arrivals	Aim: Prevent introduction of infected animals. **Control measures:** Quarantine, vaccination
Food	Aim: Prevent entry of the disease in infected food products. **Control measures:** **Wash, disinfect fruit, vegetables. Vermin proof food storage. Food storage room with easy to clean surfaces.**
Fomites (vehicles, equipment, crates, clothing and shoes etc.)	Aim: Prevent disease being transferred to animals, their food or anything they may come in direct contact with. **Control measures should disease be widespread (outbreak):** Food baths, shoe changes, hand washing.
Faeces / waste food/ soiled bedding etc.	**Control measures in the event of outbreak:** Burying organic waste, burning of bedding
Infected Humans	Prevention of transfer of a disease strain that can infect both humans and animals. • In the event of a human pandemic with a virus that is capable of causing serious disease in our animals, prevention is likely to be impossible. **Recommendations** Efforts should concentrate on keeping our staff healthy. Health awareness, general personal hygiene, education. Minimise contact with the animals and staff

Additional points:

- These contingency measures are liable to revision as the threat changes and our knowledge of the disease and its control develops. They will be reviewed on a regular basis (minimum monthly).
- The contingency of how we would operate and provide care for our animals in the event of a human pandemic is also not covered within this document.

Summary:

Measures in place (DATE):	Quarantine Pest control Hygiene
Measures to be put into effect ASAP: Timing to be supplied as soon as they are known.	Vaccination
Measures to be put in place if outbreak:	Epidemiological study Isolation of healthy from non-healthy animals

Disease of Concern – Strongyloidiasis – an alternative template to that already shown

	Non human primates	**Humans**
Who is at risk	Great Apes only. Severe if immunosuppressed	• Primate Keepers, • Vet Staff, • Maintenance Particularly those that are Immunosuppressed
Know positives at Sanctuary?	Two Chimpanzees in main enclosure	Unknown – staff not screened
Source	Contaminated soil, food items or hair/bedding. Either brought in with new animals or with soil or with food.	Contaminated soil, bedding or food, inadequate personal hygiene
Transmission	Cannot be passed directly from one individual to another but needs to go through a period of larval development usually in soil (but can be any warm dark place including fur, dirty clothing etc). L3 larvae can penetrate skin and mucus membranes (e.g. buccal mucosa)	
Clinical Signs	• Acute, relapsing or persistent diarrhoea, • Death if untreated in immunosuppressed animals.	• 95% asymptomatic. • The immune response limits the infection to the small intestine. • Massive tissue invasion can occur in

	• Persistent infection likely if left untreated	the immunosuppressed. Death usually due to secondary E.coli septicaemia.
Implications	Chronic – difficult to eradicate	Infection can last for decades –infection can be serious particularly if become immunosupressed.
Treatment	Ivermectin (repeated dosing required), albendazole (compliance issues)	Albendazole (good for even high infection), Invermectin (not effective against adult worms)
Management recommendations	6 monthly faecal checks in apes. Investigate bouts of diarrhoea longer than 48 hours duration Treat any animals testing positive.	Fact sheet issued to all staff at risk. Recommend 6 monthly faecal checks and treatment if positive via Occupational Health Service Good personal hygiene. Wear gloves and clean overalls at all times when handling animals, contaminated waste or working in enclosure
References	Wendi Bailey PhD (pers comm.), www.cdc.gov, PASA vet manual at www.pasaprimates.org, Oxford handbook of Tropical medicine	

3.3 INTRODUCTION TO INFECTIOUS DISEASE EPIDEMIOLOGY

Compiled by S.Unwin

I will never be a good epidemiologist – I cannot see the forest, I can only see the trees. It all depends on your perspective.

Epidemiology is the study of disease in populations, and thus is requires putting animals into groups. They are all individuals, and no two patients are ever exactly alike. However, there are a number of characteristics that can be used to group animals. They are either male or female, or they are of a certain age, or they are from a certain geographical region etc, and they share these characteristics. Epidemiology identifies such groups, ignoring the uniqueness of its members, and tries to determine whether this division of animals into groups tells us something more than we could have learned by merely observing each animal separately. Since epidemiology is a branch of medicine, our interest is usually to describe, analyse or understand patterns of disease in such groups. The most common situation occurs when we find one group of animals who are ill with some disease, and another group of individuals (often within the same group!) who are not. What is the difference between these groups? Is there some characteristic that seems to differ between them? As you will see, epidemiology can also be described in risk analysis terms.

Descriptive Epidemiology (Hazard Identification)

- **When do the cases of illness appear and where?**
- **What ages are they?**
- **Is there a group defining characteristic in common?**

Obviously in such descriptions it is not the individual cases that are of prime interest, but rather the collective pattern of disease that they form. Such straightforward *descriptive epidemiology* almost always reveals interesting patterns that we would not have observed if we had not collected the cases and ordered them in a structured manner.
But we all have inquisitive minds – we always want to know 'WHY?' – Why were there so many cases in a certain area? Why did it appear only adult males were affected? Why were there more cases last year than this year?

Such questions lead to the next step in the epidemiological analysis:

Analytical Epidemiology (Risk Assessment)

- **Compare the ill group with a healthy group**

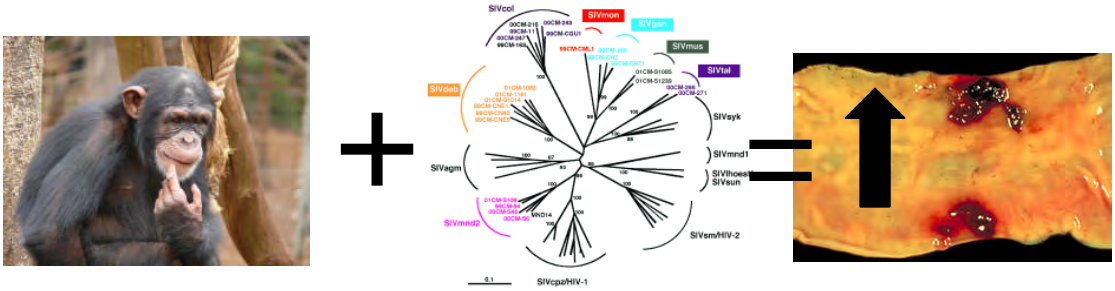


Figure 1. Animal + immunosuppression can lead to increased disease due to a secondary infection. Knowledge of these interactions is vital when attempting to manage disease issues

We test the clues offered by the descriptive study by searching for differences in characteristics between the ill and the healthy. Did the cases of gastroenteritis eat something that the others did not eat? Did the animals that contracted *Oesophagostomum* come from a different region to those who did not? Is it more common to find cases of secondary infection in chimpanzees with SIV, than in those that don't? (figure 1)
If our analytical study has been well designed, and if the clues we are investigating are appropriate, we may find strong support for a particular aetiology, a certain pathological mechanism or a certain source.

Converting Disease Knowledge into Preventative Action (Risk Management)

Figure 2. Vaccination can play a role in disease risk management

Preventative action - Can hygiene measures be instigated? Can we influence people's behaviour to reduce transmission to or from the animals? Is there a prophylactic treatment? Could a vaccine be developed? Here again, epidemiology might be called upon to evaluate the effects of preventative

measures. Did they have the effect on pattern of disease that we had hoped for?

With the exception of vaccination, all of these steps apply equally to non infectious and infectious disease epidemiology. However, there are two features that are unique to the infectious diseases.

- A case may also be a risk factor
- Animals (and people) might be immune

In a non infectious disease issue, lung cancer for example, smoking is a risk factor, and a patient with lung cancer is a case.
However, for influenza or a cold, my risk of disease during the coming winter will be greatly affected by the number of other flu cases close by.
The second feature – immunity - is also unique to infectious diseases. For most non infectious risk factors, such as toxins or radiation, there will be levels at everyone exposed will fall ill, but some cases in infectious disease will develop immunity.

These two points represent the major differences between infectious and non infectious epidemiology but there are a few more.
A case may be a source without being recognised as a case. That is, subclinical infections play an important role in the epidemiology of many infectious diseases. Ignorance of their existence would make many outbreaks and transmission chains inexplicable.
There is sometimes a need for urgency – with infectious disease outbreaks, the timeframe is sometimes closer to hours or days before some preventative measures must be decided upon. This may give little time for elaborate analyses.
Preventative measures (usually) have a good scientific basis – Often (but not always), much is known about the bacteria, viruses and other parasites that cause disease

SOME DEFINITIONS.

Incidence: Is a disease measure over time – a RATE. The number of individuals who fall ill with a certain disease during a defined time period (often 1 year), divided by the total population. For example, the incidence of TB in Cameroon may be 50 per 100 000' – or this can be shown as a percentage. Cumulative incidence refers to incidence over a longer time period – e.g. – 60% of children under 5 years old contract malaria, but we do not know exactly how the incidence has varied over these years.

Prevalence is the product of incidence and duration **at a specific time** – e.g. – the prevalence of HIV infection in several African countries is above 20 per 100 population. Prevalence is a more interesting measure for chronic or protracted diseases, as it will give some indication of the risk of exposure to others in the population.
So, a person or animal who falls ill adds 1 to the incidence of the disease. It will also add 1 to the prevalence for the duration of its disease, until it either recovers or dies. If the average daily incidence of a disease is I and the average

duration is D Days, then the average prevalence P will be: P=I x D – prevalence is the product of incidence and duration.
When comparing the incidence or prevalence of a disease between 2 populations, you must take into account the size of the groups – the denominator. For example, 3 cases of TB in a local village would give a high local incidence for that village, but may not affect the figure for national incidence very much. This concept becomes very important when trying to compare different areas on a map of disease incidence. If you don't know the denominator (population size) then you should not use the term 'incidence' at all, and refer simply to the number of cases.

Figure 3. Know your denominator – i.e. – be as precise as possible when defining the population you are investigating

Epidemiology is the study of disease in populations. Invariably, we will be analyzing subsets of a population. Thus for any meaningful analysis and management to come out of epidemiological data, the population you are talking about must be known. Thus, you must know your denominator – the population you are interested in (Figure 3). For example, if you are saying the incidence of strongyloides is 56% in chimpanzees does this mean all the chimpanzees in the world, all the chimpanzees in DR Congo, or all the chimpanzees at Tchimpounga? The implications would obviously be far reaching if you get it wrong.

Endemic Vs Epidemic

Endemic Disease – E.G – Malaria – When an infectious disease lingers at around the same incidence for a long time, it is called an endemic. There are also diseases that are endemic in one area of the world (e.g. cholera in the Ganges), which sometimes spread to other areas and become an epidemic (such as Latin America in the 1990s).
The term **epidemic** is one of the most difficult definitions of all, and many suggestions have been made. One of the shortest (and potentially therefore the best) is in the Control of Communicable Diseases Manual, namely 'the occurrences in a region of cases of an illness (or an outbreak) with a frequency clearly in excess of normal expectancy'. Some people would probably find this definition too wide, and would prefer to include something about a 'sudden rise in incidence' or 'very large number of cases', while others might want to factor in an aspect of public perception. There is just no universally useful

definition. Epidemic is also a very loaded word – many people dealing with one will therefore use the term 'outbreak'. **Pandemic**, is a worldwide epidemic – like the flu of 1918.

Note that epidemiology makes confusing use of the word 'risk'. In everyday language a risk is something that can really cause harm, but in epidemiology it just denotes the statistical likelihood of being ill if one is exposed to some factor – it says nothing about whether this factor really causes the disease. At the beginning of an analysis we do not know which factors will prove harmful, and initially they are all suspects.
The use of the word 'exposure' may be even more frustrating, especially for someone with a background in infectious diseases. Normally, when we say that someone has been exposed to an infectious agent, we mean that they have physically met the bacteria, virus etc – someone with influenza has coughed at them, or they have eaten food known to be contaminated with *Salmonella.* However, epidemiology uses the word exposure to denote to have met a risk factor for the disease, WHICH MAY OR MAY NOT BE THE CAUSE. The difference between the two definitions is sometimes subtle and sometimes confusing. Consider Hepatitis B as an example. Known risk factors for acquiring this infection include blood transfusion, sexual intercourse, or contact with infected blood or saliva (such as from fight wounds). An animal with an acute hepatitis B infection may have been exposed to a number of these risk factors (epidemiological definition), but in only one of these situations (usually – unless they were really unlucky) were they exposed to the virus (infectious disease definition). The risk factors are only causal if the media involved effectively contain active virus, but they are always 'epidemiological' risk factors, because the media are potential carriers of the virus.

Risk Factor = determinant (more modern term, still not widely used).

$$\textbf{Risk} = \frac{\textbf{Number of individuals exposed to a factor who are ill}}{\textbf{Total number of individuals who were exposed}}$$

The word 'factor' is deliberately loosely defined. It is used to denote anything that could be associated with risk of disease (a food item, another animal, a behaviour and so on).

Relative Risk:

$$\textbf{RR} = \frac{\textbf{risk in individuals exposed to a factor}}{\textbf{risk in individuals not exposed to it}}$$

E.G – effect of close contact; the effect of heavy rainfall; the effect of nutrition RR > 1 = associated with disease, RR = 1 = unlikely to have caused disease, RR <1 (close to zero) may be protective against the risk.

GIS (Geographical Information Systems) are particularly useful in indicating the relative risk of a particular disease, broadly taken as the risk to individuals exposed to a factor divided by the risk in individuals not exposed to that factor. If an exposure to something we may perceive as a risk has NOTHING TO DO with a disease, then the proportion of individuals who are ill after having had

this exposure should be the same as in those who have not had the exposure. Thus, a relative risk of around 1 means that the risk of disease was nearly equal in exposed and unexposed individuals and that that item is unlikely to have caused disease. The analysis part of a GIS can rapidly go through these relative risks and highlight those more likely to be a factor in disease spread. It can be seen however that epidemiology is RARELY an exact science!

Case Study: Gastroenteritis in a zoo group of chimpanzees – measuring relative risk.

	Gastroenteritis (5 chimps)	No Gastroenteritis (10 chimps)
Quiche	2	8
Cheese Sandwiches	4	1
Salted peanuts	3	4
Chocolate cake	1	2
Cheese dip	4	7

Fifteen chimpanzees in an un-named zoo were given a 'special Christmas dinner' of food items they don't normally have. Within 24 hours, 5 of them became ill with gastroenteritis. It had been noted by the keepers that not all the chimps had eaten all of the same items (special interest was being taken of what they ate). This is what came up….

The first column tells us that four of the five ill chimps had eaten cheese sandwiches as well as cheese dip. From the second column we can see that only one of the chimps who did not become ill had a cheese sandwich, but that most chimps in that group had a go with the cheese dip. Just from looking at this table, we gain the impression that the cheese sandwiches may have been the culprit. How can we check this?
First, we realise that the table is set up the wrong way. For example, it shows that if a chimp was ill, there was a high probability that it had had a cheese sandwich, or that if they were lucky enough to stay well, there was a strong chance they had the quiche. What we really want to know is the answer to the opposite question. What is a chimp's chance of being ill if it had eaten a cheese sandwich or if it had eaten quiche? We therefore need to rearrange the table in order to ascertain how many chimps became ill out of the total that had eaten each item…….

Gastroenteritis in a group of chimpanzees

Eaten	Ill	Total
Quiche	2	10
Cheese sarny	4	5
Salted peanuts	3	7
Chocolate cake	1	3
Cheese dip	4	11

In total, 10 chimps ate quiche, and 2 of these became ill, 5 ate cheese sandwiches, and four of them became ill, and so on. From these figures we can calculate the risk of becoming ill that is associated with eating each of these food items
So risk for quiche is 2/10
Risk for cheese sandwiches is 4/5 etc.

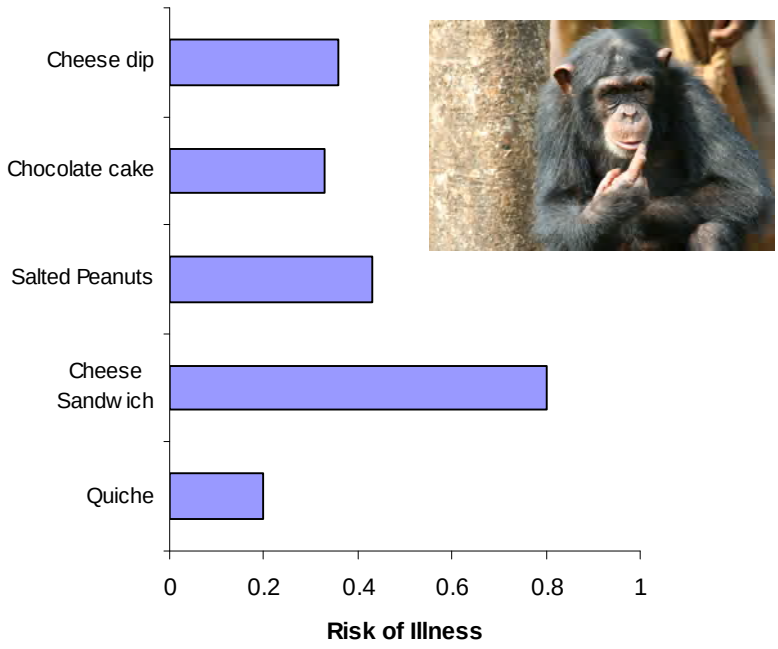


Figure 4 Risk of developing illness based on food item eaten.

The risk of illness from eating each of these items is shown in figure 4.

This gives us a list of possibly infected items, with the cheese sandwich and the salted peanuts being the most suspect, but let us just stop to think for a moment. Suppose that the Christmas dinner had nothing to do with the gastroenteritis seen in the chimps? Or that the illness was caused by a normal food item from the day before? The above result may have arisen just be chance.

Now comes the central message – **WE MUST ALSO LOOK AT THE RISK OF BEING ILL AMONG THOSE WHO DID NOT EAT THE ITEMS ON THE LIST (Relative risk).** We know that almost half of those who ate the salted peanuts were ill, but what conclusion would we draw if we found the same proportion of those who skipped the salted peanuts were ill? The chimps in this gastronomically challenging meal ate many different things, and for most of the items there will be a mixture of those who happened to eat the food and those who did not. If an item was definitely NOT a cause of the illness, we would expect the same risk of being ill whether a chimp ate it or not.

This way of thinking is fundamental to all epidemiology. If an exposure has nothing to do with a disease, then the proportion of individuals who are ill after having this exposure should be the same as in those who have not had the exposure.

We next proceed to list the outcome according to what chimps did NOT eat. From the first table, we can see that 3 of the 5 chimps who became ill did not eat quiche, and that 2 of the remaining 10 chimps who remained well also did not each quiche and so on

Gastroenteritis in a group of chimpanzees

Not Eaten	Ill	Total
Quiche	3	5
Cheese sarny	1	10
Salted peanuts	2	8
Chocolate cake	4	12
Cheese dip	1	4

So this table shows the number of chimps from the first table that became ill out of the total number who did not eat them. We can then calculate the risk of being ill if a chimp had NOT eaten each of these items ,and plot it on a graph together with the risks calculated previously……

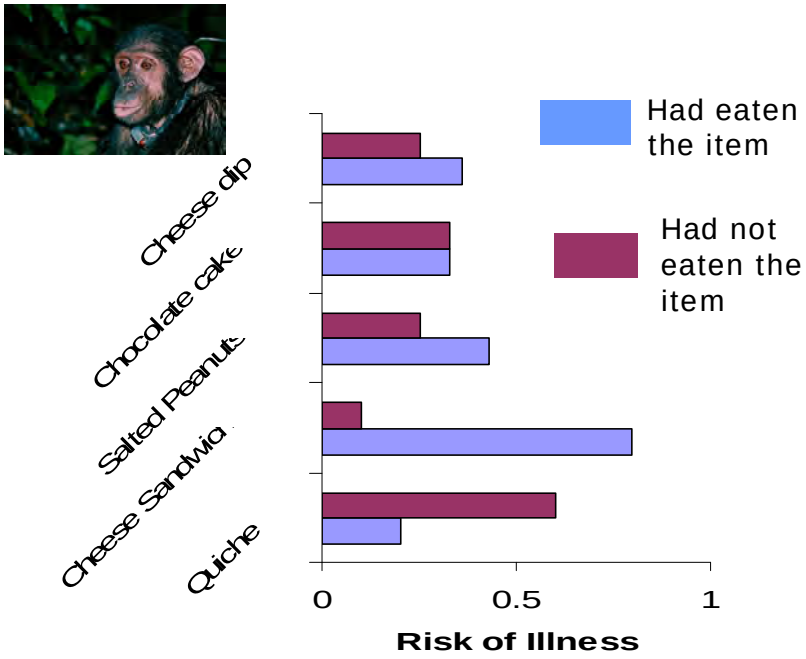


Figure 5. Risk of illness based on food item, comparing if animal had eaten that food item or had not.

Figure 5 shows that the risk of being ill is about the same whether or not a chimp ate salted peanuts, chocolate cake or cheese dip. Eating quiche seems almost to have had a protective effect, whereas cheese sandwiches are clearly the most likely culprit, with a risk of being ill of 0.8 in those who ate it, compared with a risk of 0.1 in those who did not.
The RELATIVE RISK of illness as a result of eating the different food items at the meal can be shown…..

Gastroenteritis in a group of chimpanzees – relative risk

Food	RR
Quiche	0.33
Cheese sarny	8
Salted peanuts	1.72
Chocolate cake	0.93
Cheese dip	1.44

RR = risk in individuals exposed to a factor
risk in individuals not exposed to that factor

Remember that a RR of around 1 means that the risk of disease was nearly equal in exposed and unexposed individuals and that this item is unlikely to have caused disease.

From our calculations we find that the RR of causing illness is clearly highest for the cheese sandwiches, and we can conclude with some certainty that this time they were responsible for the gastrointestinal illness.
Two questions remain. First, why was one chimpanzee ill who had not eaten cheese sandwiches, and secondly, why was one chimp well who had eaten a cheese sandwich? There are several plausible answers to these questions. Perhaps the first chimp was ill due to something else, or maybe he did eat a cheese sandwich and just wasn't observed. The second chimp may also have not been observed, but may have eaten so little of a sandwich that he did not become ill, since the risk of infection is often related to the dose of the pathogen.
One may also question why the quiche seemed to have a protective effect against illness. One possible explanation is that, even for chimps, having both quiche and cheese sandwiches was just too much, so those who ate quiche will be largely the same chimps who didn't eat the sandwiches, and who thus escaped infection.

SOME MORE DEFINITIONS:

Attack Rate: The attack rate of a disease is the number of cases, divided by the number susceptible exposed. This is the basic measure of infectivity.

Vector: An animate transmitter of infectious agents (usually an invertebrate to vertebrate transmission. A fomite is an inanimate transmitter (e.g. shoes).

Transmission route: aerosol, faecal-oral. WHAT OTHER TRANSMISSION ROUTES ARE POSSIBLE?

Zoonosis: an infection that can move from animal to human, and these days, vice versa (c.f anthropozoonosis).

Incubation period (figure 6): This is NOT a fixed number of days for any disease, but rather an interval where the middle values are more common than the extremes, and the actual period is often dependant on the infectious dose (a higher dose usually gives a shorter incubation period). The distribution of incubation periods is often skewed to the left (I.E shorter rather than longer), and references usually give the median (or mean) period, plus a minimum and maximum.
The incubation period extends from the moment an animal is infected until they develop clinical signs of disease. During this time they may be infectious, infectivity often increasing towards the end of the incubation period. This fact has important implications for disease control, since isolation of cases will often occur too late to prevent disease. THIS IS AN IMPORTANT REASON TO MAINTAIN A STRICT QUARANTINE.

Infectious period: This is the time period during which an animal can transmit disease – in many diseases there is therefore overlap with the incubation period.

Latent period: This is the time period from infection until the infectious period starts

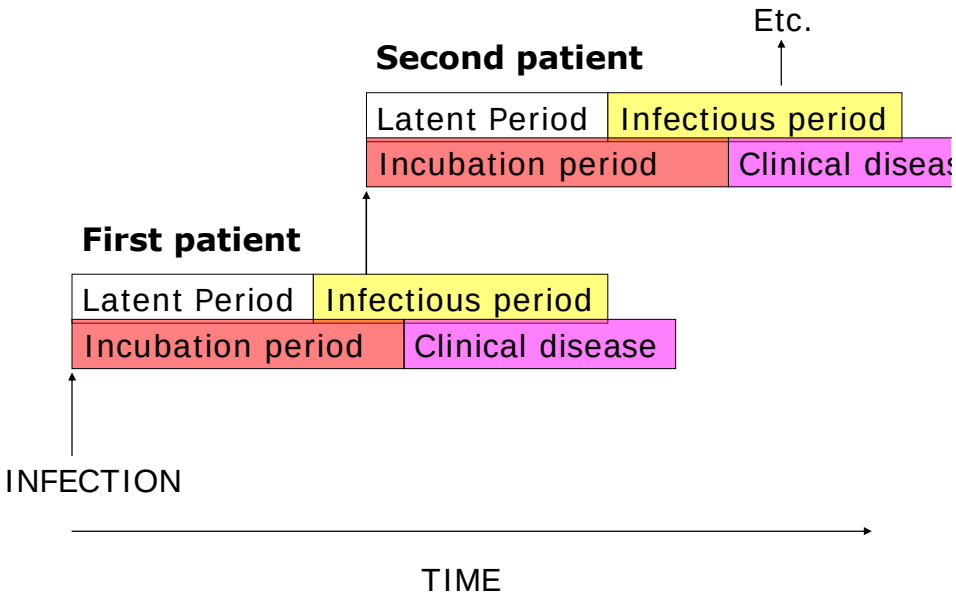


Figure 6. Phases of illness post infection

Setting up disease investigation (observational) studies

We use these sorts of studies when we don't know all the exposure details. The calculation of risks and relative risks requires a FULL KNOWLEDGE of all of those exposed and of all of the cases. Unlike the gastroenteritis example from the previous section, we will very rarely know all that we need to know for any given disease situation, for a disease analysis to be as complete as possible. This is where utilizing observational studies becomes useful.

A 2x2 table (table 1) is the cornerstone of all epidemiological research, and often the first thing one draws up when starting to investigate some data:

	Diseased Animals (Cases)	Non Diseased Animals (Controls)	Total
Hypothesised risk factor present (Exposed)	a	b	a + b
Hypothesised risk factor absent (Not Exposed)	c	d	c + d
Total	a + c	b + d	a + b + c + d = n (total)

Table 1. A 2x2 table used in epidemiological research

1. LONGITUDINAL STUDIES

The Cohort study: Rates –the concept of bias

In a cohort study, a group (cohort) of animals exposed to an hypothesized risk factor, and a group not exposed to the factor are selected and observed to record development of disease in each group. For example, if excessive moisture was considered a risk factor for pneumonia in a particular enclosure, a suitable cohort study would comprise a group of chimps housed in a very wet enclosure (exposed) and a group of chimps housed in a dry enclosure (unexposed), each of which would be monitored for the development of pneumonia. Therefore, INCIDENCE is measured and a + b and c + d are predetermined. So we are investigating rates – the number of subjects who fall ill, divided by the total time under study, added by all of the subjects in the cohort. One problem in the analysis of cohort studies is that all subjects are rarely observed for the same time period.

FURTHER THOUGHTS – how does the way a cohort study is set up influence the concept of bias? (are the subjects in the study a good sample of the population we want to make inferences about?)

In cohort studies we follow defined groups over time to see how many of them develop disease. By dividing this figure by the original number of subjects, we can calculate the actual risk of disease in each group. In real life, the subjects of a cohort study often enter it at different times, and it becomes practical to use the total time in the study to calculate what proportion fall ill per animal-time unit (months or years), which is then called a rate.
Cohort studies often take a long time, and it is important that all of the subjects are followed up for the entire study, or at least that the causes of loss to follow-up are known for each subject. If we selectively lose contact more with one group of subjects than with the other (e.g. if the animals we denote as 'losses to follow up' have in reality died from the disease we are studying), then our estimate of risk or rate will be biased.
Bias may be introduced either by the selection of subjects or by the way in which data is collected. Avoidance of bias requires careful consideration when a study is planned, as once it has been introduced, it may be impossible to adjust for it in the analysis of the data.
A controlled, randomized, double-blind trial tries to eradicate confounders (known and unknown) and biases by letting chance determine who is allocated to which group, and by precluding observer bias. If confounding variables are to be equally distributed between the groups, these cannot be too small, since chance may easily play tricks with small numbers.
The choice between performing a cohort study or a case-control study (see below) is often governed by considerations of time and money. In general, cohort studies have fewer problems with bias, but are more time consuming and expensive.

The Case-control study: Odds and odds ratios – the concept of confounding

In a case control study, a group of diseased animals (cases) and a group of non diseased animals (controls) are selected and compared with respect to presence of the hypothesized risk factor. Risk Factor = excessive dampness in the enclosure. Thus a case control study of pneumonia would involve identification of cases of pneumonia and comparison of the location of those cases (damp Vs dry environment), and a control group that do not have pneumonia. Thus a + c and b + d are predetermined. Case-control studies may be conducted with new studies and therefore may utilize BOTH incidence or prevalence values. What are the 'odds' that the risk factor has something to do with the disease?

Odds for cases = number of cases exposed to the factor
Number of cases not exposed to the factor

Odds for controls = number of controls exposed to the factor
Number of controls not exposed to the factor

Odds ratio (OR) = odds for cases
Odds for controls

FURTHER THOUGHTS – What are confounding variables? How are they related to causation? A confounder loosely describes a factor that might influence the strength of the association (I.E the magnitude of the RR or OR) between the risk factor we are currently studying and the disease. This affects causation – I.E Have we found the REAL causative factor in our study, or is there a confounder somewhere which we have not observed and which is the real cause of the disease. This is a constant problem for epidemiological research.

If we lack information about exposures and outcomes in a clearly defined population, we can conduct a case-control study. Risks cannot be calculated, but instead we use odds and odds ratios, which are based on similar ideas. Since we are only looking at a sample of all possible cases and controls, there is a statistical uncertainty in the exact figures, which is measured by calculating a confidence interval. We say that a factor is significantly associated with disease if the confidence interval around the OR does not include 1. The most difficult part of a case control study is choosing appropriate controls.
Controls should be selected not to be as similar to the cases, but rather to inform us how common a certain risk factor or exposure is in the background population from which the cases arose.
Even if a factor is significantly associated with disease, this may just be a statistical finding, where the division according to exposure also divides the individuals into high-risk and low-risk groups according to some real risk factor. This is called confounding.
The concept of confounding is closely coupled to the concept of cause, and the observational science of epidemiology will always have a problem with proving that it has found a proper cause of disease.

2. CROSS SECTIONAL STUDIES

The cross-sectional study involves the selection of a sample of *n* individuals from a larger population, and then the determination, for each individual, of the SIMULTANEOUS presence or absence of disease and hypothesized risk factor; prevalence is therefore recorded. For example, in across sectional study of pneumonia, a sample of chimps would be selected and classified according to enclosure and whether or not they had pneumonia. At the beginning of the cross sectional study, only the total number of animals (n) is predetermined. The numbers of animals with and without the disease, and possessing or not possessing the risk factor, are not known initially.

These studies are particularly useful when looking at the seroprevalence of a disease across a population (for example the prevalence of confirmed SIV in PASA sanctuaries). Such studies may be rendered more analytical by relating seroprevalence to factors such as age, gender, geographical location etc. This raises the idea of seroepidemiology (using serological factors in epidemiological studies). As well as looking at disease prevalence, these studies are also useful in determining the sensitivity and specificity of diagnostic tests, in the evaluation of vaccine effects, and to follow the incidence of an infection. This last can be achieved by sampling a defined cohort, or by means of repeated samples from a larger population. In the latter case, the incidence is estimated from changes in prevalence between samples. An advantage of seroepidemiology in this situation is that it obviates the need for continuous surveillance of cases – the cumulative incidence between two time points will be directly evident from the serological data. In addition, subclinical cases will be included.

	Advantages	**Disadvantages**
Cohort studies	1. Incidence in exposed and unexposed individuals can be calculated	1. Exposed an unexposed proportions in target population cannot be estimated
	2. Permit flexibility in choosing variables to be systematically recorded	2. Large numbers of subjects are required to study rare diseases
		3. Potentially long duration for follow up
		4. Relatively expensive to conduct
		5. Maintaining follow up is difficult
		6. Control of extraneous variables may be incomplete
Case Control Studies	1. Well suited to the study of rare diseases or of those with long incubation periods	1. Exposed an unexposed proportions in the target population cannot be estimated
	2. Relatively quick to mount and conduct	2. Rely on recall or records for information on past exposures
	3. Relatively inexpensive	3. Validation of information is difficult or sometimes impossible
	4. Requires comparatively few subjects	4. Control of extraneous variables may be incomplete
	5. Existing records can	5. Selection of an

	occasionally be used 6. No risk to subjects 7. Allow study of multiple potential causes of a disease 8. Suited to the study of interaction between genotype and environmental factors	appropriate comparison group may be difficult 6. Incidence in exposed and unexposed individuals cannot be estimated
Cross-sectional Studies	1. When a random sample of the target population is selected, disease prevalence, and proportions exposed and unexposed in the target population, can be estimated 2. relatively quick to mount and conduct 3. Relatively inexpensive 4. Current records can occasionally be used 5. No risk to subjects 6. Allow study of multiple potential causes of disease	

TABLE 2: Comparison of the advantages and disadvantages of cohort, case control and cross sectional studies (Based on Schlesselmann 1982 and Clayton and Mckeigue 2001)

COMPARISON OF THE TYPES OF STUDY

Case control studies can be conducted relatively quickly and are a useful means of initially 'trawling' for risk factors. Cohort studies, in contrast, may have a long duration (particularly those of diseases with lengthy incubation and latent periods such as cancer) and often focus on a specific risk factor. A logical requirement of demonstration of cause is that an animal is exposed to a causal factor **before** disease develops. The design of cohort studies, which resemble an experiment, ensures that this temporal sequence is detected. However, cross sectional and case control studies may NOT detect the sequence. For example, if the association between a damp environment and pneumonia was being investigated in chimps were being investigated using a cross sectional study (a damp environment being the hypothesized risk factor) then chimps with pneumonia in the damp enclosure may be identified, HOWEVER, pneumonia may have developed before the damp conditions

arose in some instances, in which case that could not have been a component cause in those animals. For this reason, and the reason that a cohort study measures incidence, the cohort study is therefore a better technique for assessing risk and identifying causes than the other two types of study.

3.4 QUARANTINE EXAMINATION PROCEDURES

W.Boardman, E.Dubois. and S.Unwin (Certain templates based on material from NEZS)

- Quarantine is the separation of newly received primates from those already in the facility until their health can be evaluated.
- The purpose of such isolation is to prevent the introduction of infectious disease to the resident population e.g. parasitic, fungal, protozoal, viral or bacterial.
- In addition, during this period new primate can become accustomed to their new diets and housing, and baseline medical data can be gathered.
- Quarantine procedures must be applied rigorously.
- An all in all out policy is the most effective procedure.
- Given that the origin of primates coming into the captive group has usually involved close contact with man for (often) prolonged periods, the potential for disease is high.
- Furthermore, the purpose of the project maybe to provide primates for release into the wild. Thus, any disease present in the captive group may be transmitted to wild primate populations with potentially devastating consequences.
- The protocols described therefore represent an ideal situation towards which the project should aim.
- Quarantine facilities should be as physically isolated as possible from the other enclosures – minimum of 20m with a physical barrier is best
- Facilities should be sited downwind and downstream of the resident groups.
- Personnel working with quarantined primates must observe established procedures to prevent cross-contamination to resident primates (see below).
- These procedures include personal hygiene, the use of separate equipment (such as feeding bottles, cleaning brushes etc), footwear and clothing for quarantined primates and the thorough disinfection of all such items after use each day.
- Ideally, staff would care for quarantined primates exclusively.
- At least contact with quarantined primates should always follow contact with resident primates and never vice-versa. (e.g. feed and clean resident primates, then feed and clean quarantined primates, then wash thoroughly.)
- Such isolation procedures should also be applied to any primates in the resident groups that becomes ill and requires treatment.
- **A minimum quarantine period of 90 days is recommended.** This is the minimum time required to test for the diseases of concern, as well as the time required for those animals infected with any disease of concern to manifest clinical signs.
- In certain circumstances it may be wise to increase this period to 6 months (e.g. for any primates which are known to have been in contact with tuberculosis).

- At the end of the quarantine period, the enclosure should be thoroughly disinfected with an appropriate disinfectant (based upon diseases detected e.g. viricidal).
- Ideally the facilities should then be unused for a period of 7-10 days.

STABILIZATION PERIOD

- A period of 1 day to 7 days (dependant on the individual primate in question) should be allowed for "settling in".
- During this time, the primate may adjust to its new environment, food preferences and behavioural patterns can be assessed and a medical record established.
- Current medical problems will require immediate attention during the first week.

FULL PHYSICAL EXAMINATION UNDER GENERAL ANAESTHESIA

- This should include an assessment of general health & condition, age, weight, dental health, external parasite burden, previous injuries etc.
- Animals should be permanently identified (e.g. subcutaneous microchip transponder)
- Refer to the Biological sampling section (3.17) but as a guide:
- Blood and serum samples should be taken for routine haematology (including screening for anaemia & blood parasites - especially malaria) and serum biochemistry, hepatitis A, B and C tests (serum) and serological testing for SIV (Simian Immunodeficiency Virus) and HIV infection. Other diseases for testing will depend on each sanctuary's diseases of concern list.
- It is also wise to submit serum/ plasma/ whole blood to test for a panel of other human and primate viruses.
- Additional serum should be taken and stored at minus 20^{o} C (preferably – 70C) for a serum bank for future reference
- Ideally cells for DNA isolation should be stored (e.g. hair follicles – which can be stored in DMSO for improved long term viability). See the section on Sample Collection.
- Haematology profile: Complete Blood Count, Fibrinogen
- Biochemistry profile:
    - Sodium Potassium
    - Urea CPK
    - Creatinine Glucose
    - ALT Total bilirubin
    - Gamma GT Alk. Phos.
    - AST Protein
    - Albumin Globulin
    - Calcium Phosphate
    - Cholesterol Triglyceride
    - LDH CPK
- Urinalysis: using a dip stick plus possible submission to laboratory for cytology

FAECAL EXAMINATION
Refer to Parasitology section 3.10

Faecal samples should be examined by direct microscopy and by a centrifugation
technique internal parasites and cultured for Salmonella, Campylobacter, Shigella and other pathogenic bacteria on at least three occasions during the quarantine period. Faeces for culture should be collected in transport media using swabs. Salmonella, Campylobacter and Yersinia should be serotyped if possible. If not, note only as carriers (I.e. don't treat) UNLESS the animal is showing signs of clinical disease. A generalised faecal culture also provides a good baseline of normal bacterial flora. Refer to section 5.5 for information on how to set up a bacterial field lab.
Where parasites have been detected and subsequently treated, further tests should be carried out during quarantine
The numbers of helminth eggs per gram of faeces examined is often directly related to the clinical severity of infestation. However, in severe diarrhoea, due to a dilution effect large numbers may not be seen. This is not the case for protozoa. Therefore, in the case of primates with diarrhoea and a high faecal protozoal count, it is wise to consider other primary causes before ascribing the problem solely to the protozoan identified

Deworming

- Ivermectin/Milbemycin/ Moxidectin should be administered every 2-4 weeks during quarantine. (However if lice or scabies are detected then dosing at 2 week intervals is preferred)
- Pyrantel/Mebendazole/Albendazole should also be given early in the quarantine period, (hookworms and ascarids may not be susceptible to Ivermectin/ Milbemycin/ Moxidectin) and if parasites are confirmed on faecal examination, treatment should be repeated in 2-3 weeks.
- Praziquantel can be used if tapeworms are confirmed.
- Deworming every 6-12 months should occur regardless for all primates.

TUBERCULOSIS TESTING
Refer to Tuberculosis section 4.3

- Should tuberculosis be introduced it may be extremely difficult to eradicate and the long-term consequences could be disastrous.
- Each primate should therefore be tested AT LEAST TWICE for tuberculosis within the quarantine period.
- If using the intradermal skin test method, there should be at least 42 days between tests to avoid false positives on subsequent testing.
- An intradermal injection of 0.1ml Mammalian Old Tuberculin (MOT) into the skin of an upper eyelid (alternating sides on successive tests) under sedation, and observation of the local reaction at 24hrs, 48hrs and 72hrs.
- A comparative test using 0.1ml of avian tuberculin 25 000 iu/ml is given intradermally in the skin of the right eyelid and 0.1ml of bovine tuberculin 25 000iu/ml is given intradermally into the skin of the left eyelid can also be employed. Observation is done at 24, 48 and 72hrs. An increased swelling on the left eyelid (bovine) in comparison to the right eye (avian) indicates a suspicious result.
- Other sites can be used like the abdomen and the medial forearm. The sites can be circled with an indelible marker.
- There is a possibility of obtaining false positive results; definitive diagnosis is therefore based on repeated positive results and further diagnostic procedures. The intra dermal comparative skin test is however a valuable tool in definitively eliminating those individuals which test negative from further suspicion.
- FOR INTRADERMAL SKIN TESTING INTERPRETATION, PLEASE REFER TO THE TUBERCULOSIS SECTION 4.3
- For other tests for tuberculosis, also refer to the tuberculosis section 4.3.
- NB: Intradermal testing of juveniles < 1 year old may not be accurate due to immaturity of the immune system.
- Incoming primates that clear these tests are considered free of tuberculosis and can be introduced into the resident group after all other quarantine procedures have been carried out.
- Primates suffering from the later stages of clinical tuberculosis may skin test negative due to an immunologically depressed state known as anergy. Thus any very thin or weak primate, or one suffering from a chronic respiratory condition should be treated with extreme caution and suspicion.
- Primate testing positive: see "Tuberculosis section 4.3 .

VACCINATION
Refer to Vaccination section 3.12

Vaccinations should be given during the quarantine period provided that the routine haematology/biochemistry described during the clinical examination demonstrates no current disease. It is important not to vaccinate against measles when TB testing

NECROPSY
Refer to Necropsy section 3.18

All primates who die during the quarantine period **must** be post mortemed (Refer to 3.18).

What follows is an example of a primate quarantine procedure, with associated suggestions on signs and labels. You can use this as a template for your own Quarantine facility.

All quarantine personnel must sign to say that they have read and fully understood the information given in this document and that they undertake to follow the working practices set out there in.

Danger of Disease

Severe and often fatal diseases can be spread between humans and animals, especially non human primates
This spread can be by direct contact, or in the air or from contamination by bodily fluids such as blood and saliva.
Infected animals and humans can incubate disease, without showing clinical signs for days or months, depending on the disease.

Purpose of quarantine

Precautions designed to
1. prevent cross infection
2. prevent escape and unauthorised entry

Quarantine Premises

All premises must be of strong permanent construction and designed so as to prevent the escape of the quarantined animals or access by other animals or unauthorised persons.
Premises must be maintained in a good state of repair, have adequate fire precautions and washing facilities for staff.
All entrances to the quarantine area must have an outer and inner door forming a trap to prevent animals escaping. Both doors must open inwards and be self closing if at all possible.
Adequate amounts of natural daylight + sufficient artificial lighting for proper cleansing of caging and safe handling of animals.
Fresh air, drinking water, cool areas/ shade and heating required (as appropriate for the species).

Quarantine Period.

This will depend on the species and diseases of concern, but must not be less than 90 days for primates or 30 days for other species.
Animals arriving within 48hrs of each other may be considered as one consignment. Another batch of animals arriving after this period will be considered as a separate consignment and must be isolated throughout the quarantine period. Discretion can be made to begin quarantine periods again, if the social requirements of the new arrivals need to be met.
Quarantine period may be extended if:

1. An outbreak of rabies is suspected or confirmed.
2. An animal sharing the same quarters dies or becomes ill towards the end of the detention period.

Quarantine Personnel

Only authorised personnel may work in the quarantine area.
These include:

1. the veterinary supervisor and authorised deputy
2. assigned keeping staff
3. a person authorised by and assisting 1 or 2 in their duties

The daily routine should allow the frequency of movement of personnel between quarantine and non quarantine areas to be minimised. This limits the potential spread of disease to non quarantine animals kept at the same sanctuary.

Protective clothing and hygiene measures

All personnel must wear the overalls and boots provided. They must be kept clean and only be worn in the quarantine areas.
Visors, masks and gloves should also be available.
A disinfectant (e.g. F10, bleach etc) should be chosen.
Separate utensils, food bowls etc should be used in quarantine and non quarantine areas.
Provision must be made for incineration of all soiled bedding and waste food if this option is available.
Arrival crates must be thoroughly disinfected before leaving the quarantine premises.

Animal Security

Animals in quarantine must not be removed from their cages unless under the express permission of the veterinarian or primary care giver. Movement will only be allowed for essential medical or welfare reasons.
Staff are obliged to report to the veterinarian, senior keeper or manager any animal escape (either from its cage or from the quarantine area).

Animal Health Care

Quarantine premises must be visited by the veterinarian or appointed deputy at least once weekly during the quarantine period. Health reports must be submitted to sanctuary management by the veterinarian at least monthly.
Animals in quarantine may only be treated by the veterinarian or the authorised deputy or deputies.
Any suspicion of illness and/or change of behaviour must be recorded in the diary by the keeping personnel and the veterinarian informed.
Animals must not be removed from the premises during the quarantine period for any purpose whatever unless previously authorised by the veterinarian, or Government veterinarian/ equivalent. This includes removal to a veterinary facility for treatment and removal after death.
Provision should be made so that clinical examination, post-mortem examination, routine medical treatment, anaesthesia and isolation of sick animals can be carried out within the quarantine area if required.

Human health: Bites and Scratches

First aid:

If you are bitten or scratched by an animal in quarantine:

1. flush and wash of wound thoroughly with water and detergent (viruses are often susceptible to detergents)
2. Apply 40-70%alcohol

Or tincture/aqueous solutions of iodine

Or 0.1% quaternary ammonium compounds (make sure all traces of soap washed away first)

Notification

You **MUST** then:

1. Record incident in the quarantine accident book
2. Notify veterinarian/ head keeper/ sanctuary manager
3. Contact medical assistance if required.

Record Keeping

Records to be available at all times

1. The licence, confiscation notice or order under which the animals were obtained.
2. In house records stating date, species and number of animals

moved into quarantine and their origin

moved out of quarantine and their destination

Born on the premises

Died on the premises

Removed from their cages for medical welfare or other essential reasons.

New arrivals added to the animals undergoing quarantine.

Medical records.

3. Accident records

Authorised Personnel (

SANCTUARY manager	_____ _____
Authorised Keepers / animal care staff	_____ _____
Veterinarian Vet Nurse (when required to assist vet staff)	_____ _____

QUARANTINE
RULES FOR WASTE AND EQUIPMENT

Waste = soiled bedding, uneaten food, etc
Equipment = tools, wheelbarrows, food dishes, bear crates etc.

- **Only take into quarantine what you absolutely need.**

- **Nothing comes out of quarantine unless:**

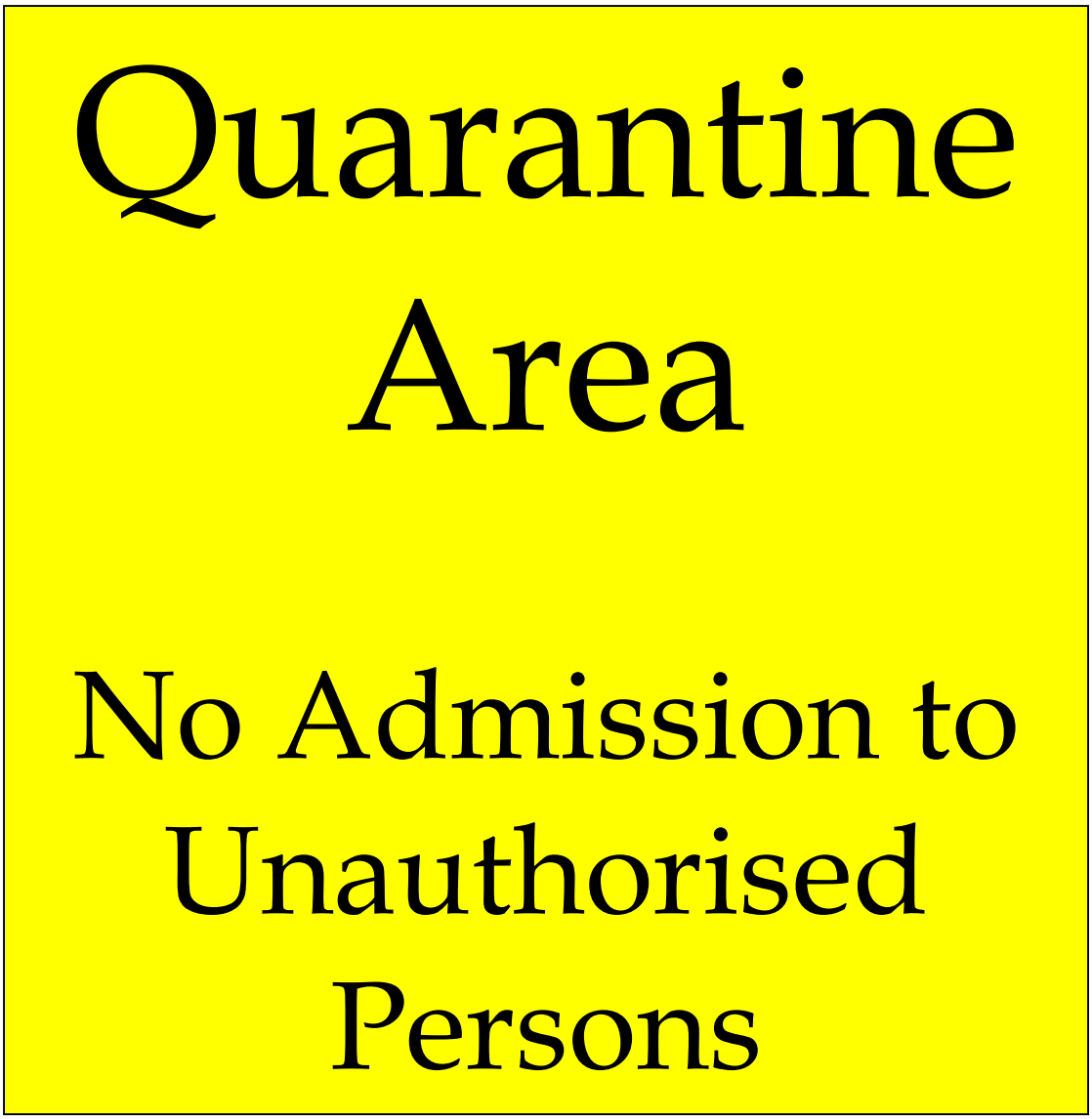
1. **It is in a bag – to be incinerated.**
2. **It is thoroughly scrubbed and then disinfected with (E.G) Virkon, bleach etc.**

Disposal of waste bags:

- **Fill bags to 2/3 full only.**
- **Tie bags with cable ties provided. Then place in a clean bag and tie this also. Write on the bags with permanent marker " infectious waste"**
- **Store bags in quarantine until It can be safely disposed of**
- **Any problems contact the Veterinarian or the sanctuary manager**

Quarantine Check List _____

Vet Responsibility		
Quarantine Procedures and Authorised Personnel		
Quarantine Sign/s		
Waste Disposal Instructions		
First Aid Instructions		
Yellow Bags and Ties		
First Aid Kit- Povidone-Iodine Surgical Scrub & Desderman N		
Foot Bath		
Animal Care Team Responsibility		
Safe Working Practices		
Training- i.e. sign off on SWPs		
Quarantine Book - for recording visitors, daily notes etc		
Accident Book		
Management responsibility		
Overalls		
Wellies		
Disinfectant		
Gloves etc.		


Quarantine
Area
No Admission to
Unauthorised
Persons

QUARNATINE SHEET

Species:	Permanent No.
Samples collected: Date to start collecting faeces: …… Day 1 [ ] Day 2 [ ] Day 3[ ]	Other ID.

Date/Time	Observation	Signature

SURGICAL SPIRIT

Warning:
HIGHLY FLAMMABLE
HARMFUL

DO NOT INHALE VAPOURS
KEEP AWAY FROM SOURCES OF HEAT AND SPARKS
AVOID CONTACT WITH BODY

PEVIDENE ANTISEPTIC SOLUTION

Povidone Iodine Solution
1% w/v available Iodine
AVOID CONTACT WITH EYES

PEVIDENE SURGICAL SCRUB

0.75% w/v available Iodine
EXTERNAL USE ONLY
AVOID CONTACT WITH EYES

SURGICAL SPIRIT

Warning:
HIGHLY FLAMMABLE
HARMFUL

DO NOT INHALE VAPOURS
KEEP AWAY FROM SOURCES OF HEAT AND SPARKS
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0.75% w/v available Iodine
EXTERNAL USE ONLY
AVOID CONTACT WITH EYES

3.5 HEALTH CHECKS

W.Boardman, E.Dubois. Reviewer S.Unwin

WHEN ARE THEY DONE?

- Three times in quarantine over 90 day period
- When being moved
- Every two years
- If contraception is required

PREPARATION

- Where and when will the examination take place?
- Do you have enough personnel to help?
- Are the caregivers informed about what you are doing and when. May need to be contacted 24 hours before?
- Have the chimps been starved prior to the anaesthetic?
- Do you have all the equipment and drugs that you need?
- Do the labs know some samples will arrive

ANAESTHESIA

- Have everything prepared? The individual should be anaesthetised for the minimum amount of time ALWAYS.
- Do you have the anaesthetic sheets?
- Review the previous anaesthetic procedures for the chimp in question. Were there any adverse reactions?
- Decide if using medicines and doses to use.
- Decide on anaesthetic regime and doses to use
- How will you administer the drugs?
- Emergency procedures – know what they are?
    - Respiratory failure
    - Cardiac failure
    - Shock
- What emergency drugs are available and do you know the doses?
    - Atropine – 0.05mg/kg IV/IM
    - Doxapram – 1-2mg/kg IV
    - Adrenaline – 0.01mg/kg IV/intra cardiac every 3-4 mins
- Are there endotracheal tubes available and an ambu bag?
- Ensure syringes and needles are available?

DRUGS TO USE

- General anaesthesia – ***least amount of drug for the shortest possible time***
    - zoletil IM,
    - medetomidine/ketamine IM,
    - diazepam PO/med/ketamine IM,
    - diazepam PO /ketamine IM,
    - ketamine IM,
    - medetomidine PO,

  - zoletil PO and
  - ketamine PO

EXAMINATION

- Weigh
- Establish intravenous access if long procedure
- Record parameters i.e. TPR, mucous membrane colour, blood pressure and oxygen saturation
- Full clinical examination.
  - i.e. teeth, ears, eyes, nose, auscultate heart and lungs, abdomen, skin, hair
- Tuberculosis testing (refer to tuberculosis section 4.3)
- Blood sample using needle or butterfly needle for serology, haematology and or biochemistry
  - Use gloves and use vacutainer and attachment
  - Femoral vein
  - Median cubital vein
  - Saphenous vein
  - Collect in EDTA tube, serum tube, citrate
- Make blood smear for examination
- Urine sample for mulitstix examination or further analysis
- Sample of faeces for examination plus or minus swab.
- Other diagnostic samples as required i.e. skin/hair/swabs.
- Radiology/ultrasound if warranted and available
- Vaccinations: e.g. Polio, Tetanus, Measles. Vaccinations will be sanctuary dependant
- Deworm for helminths and cestodes
- Treat for lice or wash coat if necessary

END OF EXAMINATION

- Give reversal drugs if necessary
- Move primate back to den
- Ensure primate is in the recovery position
- Continue to check TPR's
- Do not leave unless you are sure the chimp is about to recover i.e. has a pedal reflex, is beginning to stir
- Clear all equipment

AFTER THE EXAMINATION

- Clean all equipment and replenish used drugs, equipment etc
- Review TB reactions at 24h, 48h and 72h if using the intradermal skin test (refer to tuberculosis section 4.3)
- Send samples off for diagnostic procedures
- Spin down blood if keeping for future serology or take to lab for spinning down and storage.
- Obtain results of diagnostic samples
- Write up medical notes in detail

3.6 NUTRITION – THE BASICS

S.Unwin, A. Fidgett

GENERAL NUTRITION

Poor nutrition is often the basis of many of the ills we see in Sanctuaries. Good nutrition is based on the correct balance of nutrients. This can often be achieved by making the captive diet as similar to the diet eaten in the wild, but this is often not possible. Hence, there is a need to make feed substitutes based on nutrient content, rather than any physical similarity to feed eaten in the wild. The reader is referred to the National Research Council test Nutritional requirements of Non Human Primates, second revised edition (2003), ISBN0-309-06989-0. This chapter is designed merely to give the very basics.

The body's energy requirements are measured in calories (or joules). The following equation gives a quick estimation of how much energy an animal needs, depending on its physiological state. This is especially useful when hand raising and feeding infants.

Basal Metabolic Rate (BMR)

- BMR = 70 kcal (body weight in kg)$^{0.75}$

Maintenance

- 1.5-2 X BMR

Growth

- 3 X BMR

Reproduction

- 4-6 X BMR in the last trimester of gestation and all through lactation

Proteins. 4 to 5 kcal/g
Carbohydrates. 4 kcal/g
Fats. 9 kcal/g. Varies somewhat with the type.

Proteins

Nitrogenous compounds made up of amino acids – the nutrient. An estimate of the protein content of a feed is given as Crude Protein = N X 6.25. Microbes in the stomach of certain species and the caecum of others also provide the body with protein. Bound Protein in foods for example lignin in browse and chitin in insects, are not available to the animal.

Carbohydrates

- Simple - Sugars, Starches, Very digestible (~90%)
- Complex - Hemicellulose, Cellulose, Pectin, Gums, Chitin, Variable digestibility

Fats/ oils

Compounds made of Fatty acids – the nutrient. Relatively low proportion of diet. Highest energy per gram of food.

Vitamins
These are organic compounds, needed in the diet in minute quantities which are essential for maintenance, growth & reproduction
Fat Soluble Vitamins: Vitamins A, D, E and K. Toxicity possible, as stored in the body (except Vit E, which seems to be more rapidly lost than the others.
Water Soluble Vitamins: B Vitamins and C. Deficiency is a problem. Need in diet in sufficient quantities daily

Vitamin A. Synthesized from ß-carotene in some animals but is required as pre-formed vitamin A in Felids and possibly other carnivores. Can be toxic and antagonistic (to other fat soluble vitamins). Deficiencies can result in eye and skin problems

Vitamin D. D2 found in plant sources, D**3** found in animal sterols. New World primates, birds, reptiles, amphibians, and fish (etc.) require D3.UV light transforms precursors in the skin to active forms. Like other fat soluble vitamins, can be toxic at high levels, but is vital for calcium uptake.

Vitamin E. Suggested diet levels of 100-200 IU/kg. Deficiency is a problem for many zoo animals. Dietary requirements largely unknown, but stress induces deficiency. Uptake is dependent on captive diet

- Fresh green forages – high
- Hay - low, Fruits - very low
- Meat - lower without liver or fat

Vitamin K Synthesized by gut microbes. Deficiency is not a problem for coprophagous or pre-gastric fermenters. Warfarin (rat bait) can cause deficiency

Vitamin B complex Produced largely by bacteria. Coprophagous and pre-gastric fermenters obtain from gut microbes. Monogastrics and birds obtain from the diet and have a daily need.

Minerals
Minerals are inorganic compounds, essential for maintenance, growth & reproduction.

- Macrominerals: Ca, P, Mg, K, Na, Cl, S
- Microminerals: Mn, Fe, Zn, Cu, Co, Mo, I , Se

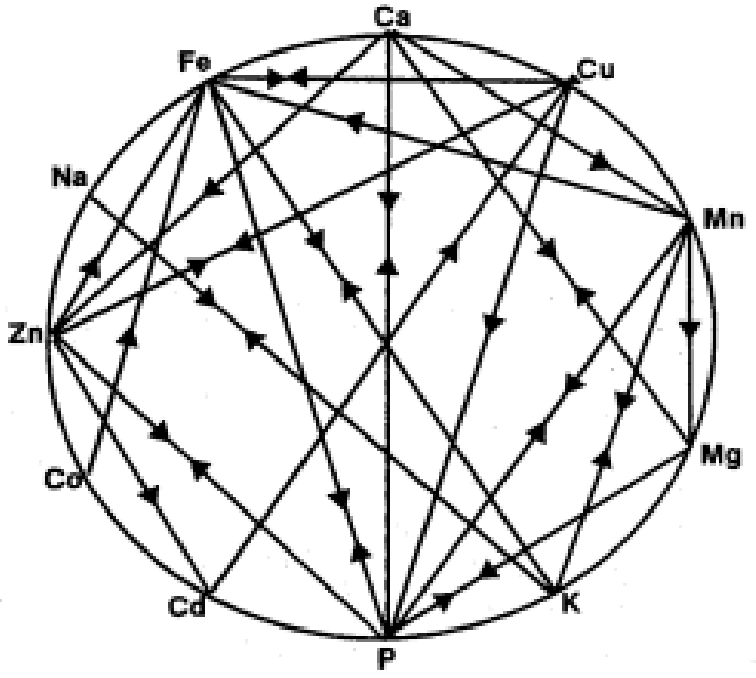


Figure 1. Interactions between some macro and micro nutrients. Double arrows indicate the interaction goes both ways. For example, Mn is needed for K uptake and vice versa. Whereas Mn is needed for Mg, Mg isn't needed for Mn. This highlights the often complex interactions between nutrients, and the need for a balanced diet.

Figure 1 highlights some of the known interactions between macro and micronutrients. For example, Calcium (Ca) and phosphorus (P) work together to form bone cells and a ratio of 2:1 is ideal for bone health. Insects lack a calcified skeleton so they lack Ca. Crickets have ratio of 1:6 (Ca:P). If feed crickets a diet high in Ca (8% of diet) they increase in Ca content. Dust crickets with calcium carbonate before feeding out increases the ratio to 1:3

PRIMATE NUTRITION

Developing primate diets depends on

- Natural History
  - Social structure
  - Feed types
  - seasonality
- Historical Diets
- Domestic Models
- Scientific Studies

Limiting steps – many primate dietary requirements are unknown, so much of the data is reliant on domestic models or humans.

Primate Dietary requirements:: Very little is known for sure

Small primates (0.5 to 5 kg): Based on Squirrel monkey
- ~ 4.0 kcal/g DM
- 23% crude protein
- 11% crude fat
- 1.1% Ca, 0.8% P

Omnivorous primates (5-30 kg): Based on WCS data
- ~ 3.6 kcal/g DM
- 18% crude protein
- 10% crude fat
- 1.0% Ca, 0.8% P

Chimps (NRC 2003)
- 14% Crude Protein
- 2.5% essential fatty acids

Proposed Diet ration - Apes
- Weigh food daily if possible
- Mimic natural diet as much as possible- especially if in field sites (figure 2 and 3)
- Be aware of diet limitations, what the animals have access to in the enclosure etc.
- No hard and fast rules

Field Observations as Basis of Diet Development – pointers to monitoring the diet of your animals:

Feeding ecology within your sanctuary
- feeding times
- identify species preferred/rejected
- Examine fecal/stomach contents
- Correlation between food handling & amounts/nutrients consumed or utilized
- Frame of reference :
    - habitat/environmental considerations
    - population/individual variability

Food/Nutrient Intake – important points to remember
- **Wt. of food offered – wt. of remains collected = amount of food consumed (roughly!). BUT**
- Diet consumed often differs from that offered, but investigating this can often be difficult, especially under field conditions, and perhaps should only be considered if a dietary deficiency has been highlighted.
    - Estimate or determine nutrients –computer–laboratory analysis
    - Digestion trials –monitor utilization


Figure 2 and 3. Gorillas are primarily folivirous (leaf eating) and there sanctuary diet should reflect this with ample browse being offered

Excess of a single item

- Toxicity
- Deficit
- Increased susceptibility to disease

Kitchen Management:

- Physical infrastructure appropriate?
- cold and dry storage guidelines
- Food preparation – done in a hygienic manner?
- Safety hazards (biological, physical, chemical)
- Quality control
- Sampling for analysis, if there is a disease problem in a group of animals

Nutrition and Disease – Disease due to dietary deficiencies manifests itself in three main situations in the sanctuary:

New arrivals:

- Period of captivity
- Previous diet
- Concurrent Disease, stress, psychological problems

Neonates and Juveniles:

- Immunocompromised
- Lack of food knowledge
- Subordinate

Bottom of the social pile:

- Diet not always balanced
- Increased stress

Enrichment (Figures 4 and 5)

- At least 10% of the diet should be in the form of enrichment. This enrichment is for situations where more than just food is required for the animals to exhibit their normal repertoire of behaviours or daily activities.
- Variety
- Feeding times
- Feeding positions
- Constant initiative

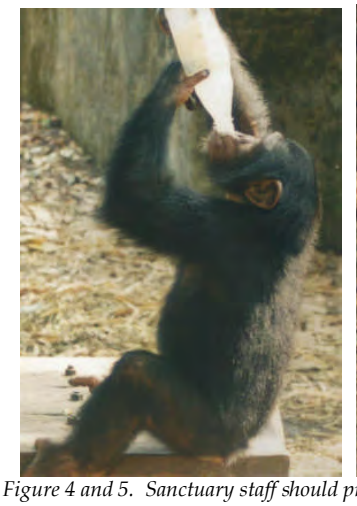



Figure 4 and 5. Sanctuary staff should provide a variety of foods, presented in a variety of ways, for their animals

Diet Manual: a recommendation for each sanctuary

- Description of feeding regimes currently in practice
- Table of food sources
- On site live food production
- Quality control issues:
    - Composition data
    - Food procurement & storage
    - Food hygiene & preparation

3.7 MANAGEMENT OF THE MALNOURISHED PRIMATE

S.Unwin (Based on WHO protocols)

INTRODUCTION

Many of the primates seen in Africa's sanctuaries have a variable degree of malnutrition. Fluid therapy in these cases forms a vital part of a successful rehabilitation in these cases. The following information is based on World Health Organisation guidelines regarding treatment of **malnourished children – Management of severe malnutrition: a manual for physicians and other senior health workers ISBN 92 4 154511 9**. This information is both relevant and thorough, and allows steps to be followed for the most successful outcome. Management of severe malnutrition is divided into three phases. These are:

- Initial treatment: life-threatening problems are identified and treated, specific deficiencies are corrected, metabolic abnormalities are reversed and feeding is begun.
- Rehabilitation: intensive feeding is given to recover most of the lost weight. Emotional and physical stimulation are increased.
- Follow-up: The continued physical, mental and emotional development of the animal must be ongoing, coupled with records of the process being maintained. A typical time-frame for the management of a primate with severe malnutrition is shown in Table 1.

Successful management of the severely malnourished animal does not require sophisticated facilities and equipment or highly qualified personnel. It does, however, require that each animal be treated with proper care and affection, and that each phase of treatment be carried out properly by appropriately trained and dedicated health workers. When this is done, the risk of death can be substantially reduced1 and the opportunity for full recovery greatly improved.

SPECIFIC NON HUMAN PRIMATE CASES

There are very few studies looking at the effects of malnutrition on non human primates. Rana et al. 2003 observed the effect of protein-energy malnutrition on the gastric and duodenal mucosa under experimental conditions. The activities of digestive enzymes (i.e. lactase, sucrase, maltase, trehalase, glucoamylase, leucine aminopeptidase, alkaline phosphatase and gamma-glutamyl transpeptidase) from the gastric (fundus, body and antrum) and duodenal mucosa [i.e. first (D1) and second (D2) part of the duodenum] were determined in 6 control, 6 protein-energy malnourished (PEM) and 6 rehabilitated young rhesus monkeys. There was a significant increase in the activity of the lactase enzyme in the antrum, and D1 and D2 portions of the duodenum of PEM monkeys, while the activity of all other enzymes was significantly increased in the D1 and D2 portions only. The increase in the activity of the above-mentioned enzymes became normal upon rehabilitation. There was no change in the enzyme activities of the gastric mucosa in mild-to-moderate PEM states. This study thus demonstrates that even mild-to-moderate malnutrition states

affect the activity of enzymes in the gastric and duodenal mucosa. Enzyme activity recovers on rehabilitation.

A syndrome of alopecia and weight loss in a colony of 10 western lowland gorillas (Gorilla gorilla gorilla) in Gabon during a 3-yr period was apparently due to a dietary protein deficiency, with nine individuals affected to some extent (Mundy et al 1998). The most severely afflicted was a 4-yr-old female who eventually died as a result of acute gastroenteritis caused by Shigella flexneri. Clinical signs included chronic alopecia, hair discoloration, failure to thrive, and weight loss, and their severity was directly correlated with the degree of hypoalbuminemia (12 g/L in the most extreme case) and normocytic normochromic anemia. Preliminary clinical tests and autopsy results suggested a dietary protein or amino acid deficiency as the cause of the hypoalbuminemia, and further analyses of serum amino acid and protein levels were consistent with a diagnosis of dietary protein deficiency. Supplementation of the colony diet with a protein preparation for humans produced a rapid amelioration of signs and improvement in body and coat condition, a normalization of serum albumin and total protein levels, and disappearance of the anemia in all affected animals except a 12-yr-old male, who responded well to treatment with anabolic steroids. The natural diet of western lowland gorillas is surprisingly high in protein, and the dietary protein requirement of captive gorillas may be increased as a result of the absence of commensal gastrointestinal ciliates.

Activity	**Initial Treatment**		**Rehabilitation**	**Follow-up**
	Days 1-2	**Days 3-7**	**Weeks 2-6**	**Weeks 7-26**
Treat or Prevent				
Hypoglycaemia				
Hypothermia				
Dehydration				
Correct electrolyte imbalance				
Treat infection				
Correct micronutrient deficiencies	Without iron		With Iron	
Begin feeding				
Increase feeding to recover lost weight ('catch-up' growth)				
Stimulate emotional and sensorial development				

Table 1. Time-frame for the management of a primate with severe malnutrition

EVALUATION OF THE MALNOURISHED PRIMATE

When first seen, the animal must be examined, a history taken and a decision made on the treatment to be given. Treatment should be started as soon as

these tasks have been completed; details of the history and examination should be recorded later.

Assessment of Nutritional Status

Severe malnutrition – see bilateral oedema

History and physical examination

A checklist for taking the animal's medical history and conducting the physical examination is given in the box below. I fully understand that most, if not all, of this information will be unavailable to you, but I think is worthwhile, so at least if the opportunity presents itself, the information is there. It helps to use a printed proforma so that the information is collected and recorded in a standard manner.

Checklist of points for taking the animal's medical history and conducting a physical examination

Medical history

- Usual/ recent diet
- Food and fluids taken in past few days
- Recent sinking of eyes
- Duration and frequency of vomiting or diarrhoea, appearance of vomit or diarrhoeal stools
- Time when urine was last passed
- Possible contact with people with measles or tuberculosis

Physical examination

- Weight and length or height
- Oedema
- Enlargement or tenderness of liver, jaundice
- Abdominal distension, bowel sounds, "abdominal splash" (a splashing sound in the abdomen)
- Severe pallor
- Signs of circulatory collapse: cold hands and feet, weak radial pulse, diminished consciousness
- Temperature: hypothermia or fever
- Thirst
- Eyes: corneal lesions indicative of vitamin A deficiency
- Ears, mouth, throat: evidence of infection
- Skin: evidence of infection or purpura
- Respiratory rate and type of respiration: signs of pneumonia or heart failure
- Appearance of faeces

Laboratory tests

Where facilities permit, the tests given in Table 2 may help to diagnose specific problems. They are not needed, however, to guide or monitor treatment. The interpretation of test results is frequently altered by malnutrition. For this reason, laboratory tests may misguide inexperienced workers. The most important guide to treatment is frequent careful assessment of the animal.

Tests that may be useful	Result and Significance
Blood glucose	Glucose concentration <54 mg/dl (3 mmol/l) is indicative of hypoglycaemia
Examination of blood smear by microscopy	Presence of malaria parasites is indicative of infection
Haemoglobin or packed-cell volume	Haemoglobin <40g/l or packed-cell volume <12% is volume indicative of very severe anaemia
Examination and culture of urine	Presence of bacteria on microscopy (or >10 leukocytes per high-power field) is indicative of infection
Examination of faeces by microscopy	Presence of blood is indicative of dysentery Presence of Giardia cysts or trophozoites are indicative of infection. Can also check for other amoebas.
Tests that are of little or no value	
Serum proteins	Not useful in management, but may guide prognosis
Electrolytes	Rarely helpful and may lead to inappropriate therapy

Table 2. Laboratory tests to check for malnutrition

INITIAL TREATMENT (DAY 1)

Principles of management

Animals with severe malnutrition are often seriously ill when they first present for treatment. Wasting, anorexia and infections are common. Successful initial management requires frequent, careful clinical evaluation and anticipation of common problems so they can be prevented, or recognized and treated at an early stage. The physiology of malnourished animals is seriously abnormal; how this affects their management is summarized in Appendix 1.
Because they are very susceptible to infection, they should, if possible, be isolated from other animals and sick humans (i.e. in quarantine). The room temperature should be kept at least 25 ^{0}C (77 ^{0}F), and eliminate draughts, to help prevent hypothermia. Intravenous infusions should be avoided except when essential, as for severe dehydration or septic shock. Intramuscular injections should be given with care, using the smallest possible gauge needle and volume of fluid.
Initial treatment lasts until the animal's condition is stable and his or her appetite has returned, which is usually after 2–7 days. If the initial phase takes longer than 10 days, the animal is failing to respond and additional measures are required. The principal tasks during initial treatment are

- To treat or prevent hypoglycaemia and hypothermia
- To treat or prevent dehydration and restore electrolyte balance
- To treat incipient or developed septic shock, if present
- To start to feed the animal

- To treat infection
- To identify and treat any other problems, including vitamin deficiency, severe anaemia and heart failure

HYPOGLYCAEMIA

All severely malnourished animals are at risk of developing hypoglycaemia (blood glucose <54 mg/dl or <3 mmol/l), which is an important cause of death during the first 2 days of treatment. Hypoglycaemia may be caused by a serious systemic infection or can occur when a malnourished animals has not been fed for a number of hours, as often happens during initial travel to the Sanctuary. To prevent hypoglycaemia in the baby, it should be fed at least every 2 or 3 hours day and night.

Signs of hypoglycaemia include low body temperature (<36.5 °C), lethargy, limpness and loss of consciousness. Often, the only sign before death is drowsiness. If hypoglycaemia is suspected, treatment should be given *immediately without laboratory confirmation*; it can do no harm, even if the diagnosis is incorrect. If the patient is conscious or can be roused and is able to drink, give 50 ml of 10% glucose or sucrose, or give F-75, (see formula below) diet by mouth, whichever is available most quickly. If only 50% glucose solution is available, dilute one part to four parts of sterile water. Stay with the animal until it is fully alert. If the animal is losing consciousness, cannot be aroused or has convulsions, give 5 ml/kg of body weight of sterile 10% glucose intravenously (IV), followed by 50 ml of 10% glucose or sucrose by nasogastric (NG) tube. If IV glucose cannot be given immediately, give the NG dose first. When the animal regains consciousness, immediately begin giving F-75 diet or glucose in water (60 g/l). Continue frequent oral or NG feeding with F-75 diet to prevent a recurrence.

All malnourished animals with suspected hypoglycaemia should also be treated with broad-spectrum antimicrobials for serious systemic infection.

HYPOTHERMIA

Infants under 12 months and those with large areas of damaged skin or serious infections are highly susceptible to hypothermia. If the rectal temperature is below 35.5 °C (95.9 °F) or the underarm temperature is below 35.0 °C (95.0 °F), the animal should be warmed. Either use the "kangaroo technique" by placing the infant on the carer's bare chest or abdomen (skin-to-skin) and covering both of them, or cover with a warmed blanket and place an incandescent lamp over, but not touching, the animal's body. Fluorescent lamps are of no use and hot water bottles are potentially dangerous. The rectal temperature must be measured every 30 minutes during rewarming with a lamp, as the child may rapidly become hyperthermic. The underarm temperature is not a reliable guide to body temperature during rewarming. All hypothermic children must also be treated for hypoglycaemia and for serious systemic infection.

DEHYDRATION AND SEPTIC SHOCK

Dehydration and septic shock are difficult to differentiate in an animal with severe malnutrition. Signs of hypovolaemia are seen in both conditions, and progressively worsen if treatment is not given. Dehydration progresses from "some" to "severe", reflecting 5–10% and >10% weight loss, respectively, whereas septic shock progresses from "incipient" to "developed", as blood flow to the vital organs decreases. Moreover, in many cases of septic shock there is a history of diarrhoea and some degree of dehydration, giving a mixed clinical picture.

Diagnosis

Many of the signs that are normally used to assess dehydration are unreliable in an animal with severe malnutrition, making it difficult or impossible to detect dehydration reliably or determine its severity. Moreover, many signs of dehydration are also seen in septic shock (see list below). This has two results

- Dehydration tends to be over diagnosed and its severity overestimated; and
- It is often necessary to treat the animal for both dehydration and septic shock

The clinical features of dehydration and septic shock are compared in Table 3 and 4.

Signs of dehydration and/or septic shock that are reliable in an animal with severe malnutrition	
History of diarrhoea	An animal with dehydration should have a history of watery diarrhoea. Small mucoid stools are commonly seen in severe malnutrition, but do not cause dehydration**. An animal with signs of dehydration, but without watery diarrhoea, should be treated as having septic shock.**
Thirst	Drinking eagerly is a reliable sign of "some" dehydration. In infants this may be expressed as restlessness. Thirst is *not* a symptom of septic shock.
Hypothermia	When present, this is a sign of serious infection, including septic shock. It is *not* a sign of dehydration.
Sunken eyes	These are a helpful sign of dehydration, but only when the sunken appearance is recent.
Weak or absent radial pulse	This is a sign of shock, from either severe dehydration or sepsis. As hypovolaemia develops, the pulse rate increases and the pulse becomes weaker. If the pulse in the carotid, femoral or brachial artery is weak, the animal is at risk of dying and must be treated urgently.
Cold hands and feet	This is a sign of both severe dehydration and septic shock. It should be assessed with the back of the hand.
Urine flow	Urine flow diminishes as dehydration or septic shock worsens. In severe dehydration or fully developed septic shock, no urine is formed
Signs of dehydration that are *not reliable* include	
Mental state	A severely malnourished animal is usually apathetic when left alone and irritable when handled. As dehydration worsens, the animal progressively loses consciousness. Hypoglycaemia, hypothermia and septic shock also cause reduced consciousness.
Mouth, tongue and tears	The salivary and lacrimal glands are atrophied in severe malnutrition, so the animal usually has a dry mouth and absent tears. Breathing through the mouth also makes the mouth dry.
Skin elasticity	The loss of supporting tissues and absence of subcutaneous fat make the skin thin and loose. It flattens very slowly when pinched, or may not flatten at all. Oedema, if present, may mask diminished elasticity of the skin.
Additional signs of septic shock	
Incipient septic shock	The animal is usually limp, apathetic and profoundly anorexic, but is neither thirsty nor restless.
Developed septic shock	The superficial veins, such as the external jugular and scalp veins, are dilated rather than constricted. The veins in the lungs may also become engorged, making the lungs stiffer than normal. For this reason the animal may groan, grunt, have a shallow cough and appear to have difficulty breathing. As shock worsens, kidney, liver, intestinal or cardiac failure may occur. There may be vomiting of blood mixed with stomach contents ("coffee-ground vomit"), blood in the stool, and abdominal distension with "abdominal splash"; intestinal fluid may be visible on X-ray. When an animal reaches this stage, survival is unlikely.

Table 3. Signs of dehydration and/or septic shock that are reliable and those that aren't in an animal with severe malnutrition

Clinical Sign	Some dehydration	Severe dehydration	Incipient septic shock	Developed septic shock
Watery diarrhoea	Yes	Yes	Yes or no	Yes or no
Thirst	Drinks eagerly	Drinks poorly	No	No
Hypothermia	No	No	Yes or no	Yes or no
Weak or absent radial pulse	No	Yes	Yes	Yes
Cold hands and feet	No	Yes	Yes	Yes
Urine flow	Yes	No	Yes	No
Mental state	Restless, irritable	Lethargic, comatose	Apathetic	Lethargic
Hypoglycaemia	Sometimes	Sometimes	Sometimes	Sometimes

Table 4. Comparison of clinical signs of dehydration and septic shock in the severely malnourished animal

TREATMENT OF DEHYDRATION

Whenever possible, a dehydrated animal with severe malnutrition should be rehydrated orally. IV infusion easily causes overhydration and heart failure and should be used *only* when there are definite signs of shock.

Oral Rehydration Salts (ORS) Solution for Severely Malnourished Primates
Because severely malnourished animals are deficient in potassium and have abnormally high levels of sodium, the oral rehydration salts (ORS) solution should contain less sodium and more potassium than the standard WHO recommended solution. Magnesium, zinc and copper should also be given to correct deficiencies of these minerals. The composition of the recommended ORS solution for severely malnourished **children** (ReSoMal) is given in Table 7. ReSoMal is available commercially. However, ReSoMal can also be made by diluting one packet of the standard WHO-recommended ORS in 2 litres of water, instead of 1 litre, and adding 50 g of sucrose (25 g/l) and 40 ml (20 ml/l) of mineral mix solution1 (see Appendix 2).

Amount of ReSomal to Give
Between 70 and 100 ml of ReSoMal per kg of body weight is usually enough to restore normal hydration. Give this amount over 12 hours, starting with 5 ml/kg every 30 minutes for the first 2 hours orally or by NG tube, and then 5–10 ml/kg per hour. This rate is slower than for animals who are not severely malnourished. Reassess the animal *at least* every hour. The exact amount to give should be determined by how much the animal will drink, the amount of ongoing losses in the stool, and whether the animal is vomiting and has any signs of overhydration, especially signs of heart failure. ReSoMal should be stopped if:

- The respiratory and pulse rates increase
- The jugular veins become engorged; or

- There is increasing oedema (e.g. puffy eyelids).

Rehydration is completed when the animal is no longer thirsty, urine is passed and any other signs of dehydration have disappeared. Fluids given to maintain hydration should be based on the animal's willingness to drink and, if possible, the amount of ongoing losses in the stool. As a guide, chimpanzees under 2 years should be given 50–100 ml (between one-quarter and one-half of a large cup) of ReSoMal after each loose stool, while older chimps should receive 100–200 ml. Continue this treatment until diarrhoea stops.

How to Give Resomal

Animals who can drink may be given the required amount as sips or by spoon every few minutes. However, malnourished animals are weak and quickly become exhausted, so they may not continue to take enough fluid voluntarily. If this occurs, the solution should be given by NG tube at the same rate. An NG tube should be used in all weak or exhausted animals, and in those who vomit, have fast breathing1 or painful stomatitis.

Intravenous Rehydration

The only indication for IV infusion in a severely malnourished animal is circulatory collapse caused by severe dehydration or septic shock. Use one of the following solutions (in order of preference):

- half-strength Darrow's solution with 5% glucose (dextrose)
- Lactated Ringers (LRS) solution with 5% glucose 1:1
- 0.45% (half-normal) saline with 5% glucose 1:1

Give 15 ml/kg IV over 1 hour and monitor the animal carefully for signs of overhydration. While the IV drip is being set up, also insert an NG tube and give ReSoMal through the tube (10 ml/kg per hour). Reassess the animal after 1 hour. If the animal is severely dehydrated, there should be an improvement with IV treatment and his or her respiratory and pulse rates should fall. In this case, repeat the IV treatment (15 mg/kg over 1 hour) and then switch to ReSoMal orally or by NG tube (10 ml/kg per hour) for up to 10 hours. If the animal fails to improve after the first IV treatment and his or her radial pulse is still absent, then assume that the animal has septic shock and treat accordingly (see previously).

Composition of oral rehydration salts solution for severely malnourished children (ReSoMal)	
Component	**Concentration (mmol/l)**
Glucose	125
Sodium	45
Potassium	40
Chloride	70
Citrate	7
Magnesium	3
Zinc	0.3
Copper	0.045
Osmolarity	300

Table 5. Composition of oral rehydration salts solution for severely malnourished children (ReSoMal)

FEEDING DURING REHYDRATION

Begin to give the F-75 diet**,** (See table 6) as soon as possible, orally or by NG tube, usually within 2–3 hours after starting rehydration . If the animal is alert and drinking, give the F-75 diet immediately, even before rehydration is completed. Usually the diet and ReSoMal are given in alternate hours. If the animal vomits, give the diet by NG tube. When the animal stops passing watery stools, continue feeding, as described later.

TREATMENT OF SEPTIC SHOCK

All severely malnourished animals with signs of incipient or developed septic shock should be treated for septic shock. This includes especially animals with:

- Signs of dehydration, but without a history of watery diarrhoea;
- Hypothermia or hypoglycaemia;
- Oedema and signs of dehydration.

Every animal with septic shock should *immediately* be given broad-spectrum antibiotics and be kept warm to prevent or treat hypothermia. The animal should not be handled any more than is essential for treatment. Nor should the animal be washed or bathed; after the animal has defecated, his or her bottom can be cleaned with a damp cloth. Iron supplements should *not* be given in the first 7 days as this may be toxic, especially if the animal is anaemic, plus may reduce resistance to infection. Other treatment is described below.

Incipient Septic Shock

The animal should be fed promptly to prevent hypoglycaemia, using the F-75 diet with added mineral mix. As these animals are nearly always anorexic, the diet must be given by NG tube. The amounts to be given and frequency of feeding are described later.

Developed Septic Shock

Begin IV rehydration immediately, using one of the fluids listed above. Give 15 ml/kg per hour. Observe the child carefully (every 5–10 minutes) for signs of overhydration and congestive heart failure (see section 4.7). As soon as the radial pulse becomes strong and the child regains consciousness, continue rehydration orally or by NG tube as described previously. If signs of congestive heart failure develop or the animal does not improve after 1 hour of IV therapy, give a blood transfusion (10ml/kg slowly over at least 3 hours). If blood is not available, give plasma. If there are signs of liver failure (e.g. purpura, jaundice, enlarged tender liver), give a single dose of 1mg of vitamin K1 intramuscularly.

During the blood transfusion, nothing else should be given, so as to minimize the risk of congestive heart failure. If there is any sign of congestive heart failure (e.g. distension of the jugular veins, increasing respiratory rate or respiratory distress), give a diuretic and slow the rate of transfusion. Steroids, epinephrine or nikethamide are of no value and should *never* be used.
After the transfusion, begin to give F-75 diet by NG tube. If the animal develops increasing abdominal distension or vomits repeatedly, give the diet more slowly. If the problem does not resolve, stop feeding the animal and give one of the fluids listed by IV infusion at a rate of 2–4 ml/kg per hour. Also give 2 ml of 50% magnesium sulfate solution intramuscularly (IM) – magnesium is essential for potassium retention.

DIETARY TREATMENT

Baby animals who do not require other emergency treatment, especially for hypothermia, dehydration or septic shock, should immediately be given a formula diet.

Formula Diets for Severely Malnourished Children

Almost all severely malnourished primates have infections, impaired liver and intestinal function, and problems related to imbalance of electrolytes when first brought to a Sanctuary. Because of these problems, they are unable to tolerate the usual amounts of dietary protein, fat and sodium. It is important, therefore, to begin feeding these animals with a diet that is low in these nutrients, and high in carbohydrate. The daily nutrient requirements of severely malnourished **children** are given in Appendix 3.

Two formula diets, F-75 and F-100, are used for severely malnourished children. F-75 (75 kcal or 315 kJ/100 ml), is used during the initial phase of treatment, while F-100 (100 kcal or 420 kJ/100 ml) is used during the rehabilitation phase, after the appetite has returned. These formulas can easily be prepared from the basic ingredients: dried skimmed milk, sugar, cereal flour, oil, mineral mix and vitamin mix (see Table 6). They are also commercially available as powder formulations that are mixed with water.

The mineral mix supplies potassium, magnesium and other essential minerals (see Table 7); it *must* be added to the diet. The potassium deficit, present in all malnourished **children**, adversely affects cardiac function and gastric

emptying. Magnesium is essential for potassium to enter cells and be retained. The mineral mix does not contain iron as this is withheld during the initial phase.

Ingredient	Amount	
	F-75a–d	**F-100e,f**
Dried skimmed milk	25 g	80 g
Sugar	70 g	50 g
Cereal flour	35 g	--
Vegetable oil	27 g	60 g
Mineral mix	20 ml	20 ml
Vitamin mix	140mg	140mg
Water to make	1000 ml	1000 ml

Table 6. Preparation of F-75 and F-100 diets

To prepare the F-75 diet, add the dried skimmed milk, sugar, cereal flour and oil to
some water and mix. Boil for 5–7 minutes. Allow to cool, then add the mineral mix
and vitamin mix and mix again. Make up the volume to 1000 ml with water. A comparable formula can be made from 35 g of whole dried milk, 70 g of sugar, 35 g of cereal flour, 17 g of oil, 20 ml of mineral mix, 140 mg of vitamin mix and water to make 1000 ml. Alternatively, use 300 ml of fresh cows' milk, 70 g of sugar, 35 g of cereal flour, 17 g of oil, 20 ml of mineral mix, 140 mg of vitamin mix and water to make 1000 ml.

Isotonic versions of F-75 (280 mOsmol/l), which contain maltodextrins instead of cereal flour and some of the sugar and which include all the necessary micronutrients, are available commercially.

If cereal flour is not available or there are no cooking facilities, a comparable formula can be made from 25 g of dried skimmed milk, 100 g of sugar, 27 g of oil, 20 ml of mineral mix, 140 mg of vitamin mix and water to make 1000 ml. However, this formula has a high osmolarity (415 mOsmol/l) and may not be well tolerated by all children, especially those with diarrhoea.

To prepare the F-100 diet, add the dried skimmed milk, sugar and oil to some warm boiled water and mix. Add the mineral mix and vitamin mix and mix again. Make up the volume to 1000 ml with water.

A comparable formula can be made from 110 g of whole dried milk, 50 g of sugar, 30 g of oil, 20 ml of mineral mix, 140 mg of vitamin mix and water to make 1000 ml. Alternatively, use 880 ml of fresh cows' milk, 75 g of sugar, 20 g of oil, 20 ml of mineral mix, 140 mg of vitamin mix and water to make 1000 ml.

If only small amounts of feed are being prepared, it will not be feasible to prepare the vitamin mix because of the small amounts involved. In this case, give a proprietary multivitamin supplement. Alternatively, a combined mineral and vitamin mix for malnourished children is available commercially and can be used in the above diets.

Constituent	Amount per 100ml	
	F-75	**F-100**
Energy	75kcalth (315 kJ)	100kcalth (420 kJ)
Protein	0.9 g	2.9 g
Lactose	1.3 g	4.2 g
Potassium	3.6mmol	5.9mmol
Sodium	0.6mmol	1.9mmol
Magnesium	0.43mmol	0.73mmol
Zinc	2.0mg	2.3mg
Copper	0.25mg	0.25mg
	Percentage of energy from	
protein	5%	12%
Fat	32%	53%
Osmolarity	333mOsmol/l	419mOsmol/l

Table 7. Composition of F-75 and F-100 diets

FEEDING ON ARRIVAL AT THE SANCTUARY

To avoid overloading the intestine, liver and kidneys, it is essential that food be given frequently and in small amounts. Animals who are unwilling to eat should be fed by NG tube (do *not* use IV feeding). Animals who can eat should be given the diet every 2, 3 or 4 hours, day and night. If vomiting occurs, both the amount given at each feed and the interval between feeds should be reduced.
The F-75 diet should be given to all animals during the initial phase of treatment. The animals should be given at least 80 kcal or 336 kJ/kg, but no more than 100 kcal or 420 kJ/kg per day. If less than 80 kcal or 336 kJ/kg per day are given, the tissues will continue to be broken down and the animal will deteriorate. If more than 100 kcal or 420 kJ/kg per day are given, the animal may develop a serious metabolic imbalance.

Table 8 shows the amount of diet needed at each feed to achieve an intake of 100 kcal or 420 kJ/kg per day. For example, if a chimp weighing 7.0 kg is given the F-75 diet every 2 hours, each feed should be 75 ml. During the initial phase of treatment, maintain the volume of F-75 feed at 130 ml/kg per day, but gradually decrease the frequency of feeding and increase the volume of each feed until you are giving the animal feeds 4-hourly (6 feeds per day).
Nearly all malnourished animals have poor appetites when first brought to a Sanctuary. Patience and coaxing are needed to encourage the animal to complete each feed. The animal should be fed from a cup and spoon; feeding bottles should *never* be used, even for very young infants, as they are an important source of infection. Animals who are very weak may be fed using a dropper or a syringe.

NASOGASTRIC FEEDING

Despite coaxing and patience, many animals will not take sufficient diet by mouth during the first few days of treatment. Common reasons include a very poor appetite, weakness and painful stomatitis. Such animals should be fed using a NG tube. However, NG feeding should end as soon as possible. At each feed, the animal should first be offered the diet orally. After the animal

has taken as much as they want, the remainder should be given by NG tube. The NG tube should be removed when the animal is taking three-quarters of the day's diet orally, or takes two consecutive feeds fully by mouth. If over the next 24 hours the animal fails to take 80 kcal or 336 kJ/kg, the tube should be reinserted. If the animal develops abdominal distension during NG feeding, give 2 ml of a 50% solution of magnesium sulfate IM to enhance potassium uptake.

The NG tube should always be aspirated before fluids are administered. It should also be properly fixed so that it cannot move to the lungs during feeding. NG feeding should be done by experienced staff.

	Volume of F-75 per feed (ml)		
Weight of child (kg)	**Every 2 hours (12 feeds)**	**Every 3 hours (8 feeds)**	**Every 4 hours (6 feeds)**
2.0	20	30	45
2.2	25	35	50
2.4	25	40	55
2.6	30	45	55
2.8	30	45	60
3.0	35	50	65
3.2	35	55	70
3.4	35	55	75
3.6	40	60	80
3.8	40	60	85
4.0	45	65	90
4.2	45	70	90
4.4	50	70	95
4.6	50	75	100
4.8	55	80	105
5.0	55	80	110
5.2	55	85	115
5.4	60	90	120
5.6	60	90	125
5.8	65	95	130
6.0	65	100	130
6.2	70	100	135
6.4	70	105	140
6.6	75	110	145
6.8	75	110	150
7.0	75	115	155
7.2	80	120	160
7.4	80	120	160
7.6	85	125	165
7.8	85	130	170
8.0	90	130	175
8.2	90	135	180
8.4	90	140	185
8.6	95	140	190
8.8	95	145	195
9.0	100	145	200
9.2	100	150	200
9.4	105	155	205
9.6	105	155	210
9.8	110	160	215
10.0	110	160	220
All rounded to the nearest 5ml			

Table 8. Determining the amount of diet to give at each feed to achieve a daily intake of 100 kcal or 420 kJ/kg

FEEDING AFTER THE APPETITE IMPROVES

If the animal's appetite improves, treatment has been successful. The initial phase of treatment ends when the animal becomes hungry. This indicates that infections are coming under control, the liver is able to metabolize the diet, and other metabolic abnormalities are improving. The animal is now ready to begin the rehabilitation phase. This usually occurs after 2–7 days. Some animals with complications may take longer, whereas others are hungry from the start and can be transferred quickly to F-100. Nevertheless, the transition should be gradual to avoid the risk of heart failure which can occur if animals suddenly

consume large amounts of feed. Replace the F-75 diet with an equal amount of F-100 for 2 days before increasing the volume offered at each feed. It is important to note that it is the animal's appetite and general condition that determine the phase of treatment and *not* the length of time since arrival.

MILK INTOLERANCE

Clinically significant milk intolerance is unusual in severely malnourished **children**. Intolerance should be diagnosed *only* if copious watery diarrhoea occurs promptly after milk-based feeds (e.g. F-100) are begun, the diarrhoea clearly improves when milk intake is reduced or stopped, and it recurs when milk is given again. Other signs include acidic faeces (pH <5.0) and the presence of increased levels of reducing substances in the faeces. In such cases, the milk should be partially or totally replaced by yoghurt or a commercial lactose-free formula.

RECORDING FOOD INTAKE

The type of feed given, the amounts offered and taken, and the date and time must be recorded accurately after each feed. If the animal vomits, the amount lost should be estimated in relation to the size of the feed (e.g. a whole feed, half a feed), and deducted from the total intake. Once a day the energy intake for the past 24 hours should be determined and compared with the animal's weight. If the daily intake is less than 80 kcal or 336 kJ/kg, the amount of feed offered should be increased. If more than 100 kcal or 420 kJ/kg have been given, the amount of feed offered should be reduced.

INFECTIONS

Anthelmintic, such as mebendazole or ivermectin, should be administered routinely

Bacterial Infections

Nearly all severely malnourished animals have bacterial infections when first arriving at a Sanctuary. Many have several infections caused by different organisms. Infection of the lower respiratory tract is especially common. Although signs of infection should be carefully looked for when the animal is evaluated, they are often difficult to detect. Unlike well-nourished animals, who respond to infection with fever and inflammation, malnourished animals with serious infections may only become apathetic or drowsy. Early treatment of bacterial infections with effective antimicrobials improves the nutritional response to feeding, prevents septic shock and reduces mortality. Because bacterial infections are common and difficult to detect, all animals with severe malnutrition should routinely receive broad-spectrum antimicrobial treatment when first arriving for care. Each institution should have a policy on which antimicrobials to use. These are divided into those used for *first-line* treatment, which are given routinely to all severely malnourished animals, and those used for *second-line* treatment, which are given when an individual is not improving or a specific infection is diagnosed. Although local resistance patterns of

important bacterial pathogens and the availability and cost of the antimicrobials will determine the policy, a suggested scheme is given below.

First-line treatment

Animals with no apparent signs of infection and no complications should be given cotrimoxazole (25 mg of sulfamethoxazole, 5 mg of trimethoprim/kg) orally twice daily for 5 days. Animals with complications (septic shock, hypoglycaemia, hypothermia, skin infections, respiratory or urinary tract infections, or who appear lethargic or sickly) should be given

- Ampicillin, 50mg/kg IM or IV every 6 hours for 2 days, followed by amoxicillin, 15mg/kg orally every 8 hours for 5 days (if amoxicillin is unavailable, give ampicillin, 25mg/kg orally every 6 hours) *and*
- Gentamicin, 7.5 mg/kg IM or IV once daily for 7 days

Second-line treatment

If the animal fails to improve within 48 hours, *add* chloramphenicol, 25mg/kg IM or IV every 8 hours (or every 6 hours if meningitis is suspected) for 5 days. The duration of treatment depends on the response and nutritional status of the individual. Antimicrobials should be continued for at least 5 days. If anorexia still persists after 5 days of treatment, give the animal another 5-day course. If anorexia still persists after 10 days of treatment, reassess the animal fully. Examine the animal for specific infections and potentially resistant organisms, and check that vitamin and mineral supplements have been correctly given.
If specific infections are detected for which additional treatment is needed, for example dysentery, malaria or intestinal helminthiasis, this should also be given. Tuberculosis is common in the human population, but antituberculosis drugs should be given only when tuberculosis is diagnosed.

Note: Some institutions routinely give malnourished children metronidazole, 7.5 mg/kg every 8 hours for 7 days, in addition to broad-spectrum antimicrobials. However, the efficacy of this treatment has not been established by clinical trials.

If fever is present (body temperature >39.5 °C or 103 °F), antipyretics should be given.

VITAMIN DEFICIENCIES

Vitamin A Deficiency

Severely malnourished animals are at high risk of developing blindness due to vitamin A deficiency. For this reason a large dose of vitamin A should be given routinely to all malnourished primates on day 1, unless there is definitive evidence that a dose has been given during the past month. The dose is as follows: 1 50 000 International Units (IU) orally for infants 6 months of age, 100 000 IU orally for infants 6–12 months of age and 200000 IU orally for animals 12 months of age. If there are any clinical signs of vitamin A deficiency (e.g. night blindness, conjunctival xerosis with Bitot's spots, corneal xerosis or ulceration,

or keratomalacia), a large dose should be given on the first 2 days, followed by a third dose at least 2 weeks later . Oral treatment is preferred, except at the beginning in animals with severe anorexia, oedematous malnutrition or septic shock, who should be given IM treatment. For oral treatment, oil-based preparations are preferred, but water-miscible formulations may be used if oil-based formulations are not available. Only water-miscible formulations should be used for IM treatment. Great care must be taken during examination of the eyes, as they easily rupture in animals with vitamin A deficiency. The eyes should be examined gently for signs of xerophthalmia, corneal xerosis and ulceration, cloudiness and keratomalacia. If there is ocular inflammation or ulceration, protect the eyes with pads soaked in 0.9% saline. Tetracycline eye drops (1%) should be instilled four times daily until all signs of inflammation or ulceration resolve. Atropine eye drops (0.1%) should also be applied and the affected eye(s) should be bandaged, as scratching with a finger can cause rupture of an ulcerated cornea.

Other Vitamin Deficiencies

All malnourished primates should receive 5 mg of folic acid orally on day 1 and then 1mg orally per day thereafter. Many malnourished **children** are also deficient in riboflavin, ascorbic acid, pyridoxine, thiamine and the fat-soluble vitamins D, E and K. There is no reason to suspect non human primates would be any different. All diets should be fortified with these vitamins by adding the vitamin mix (see Appendix 2).

VERY SEVERE ANAEMIA

If the haemoglobin concentration is less than 40 g/l or the packed-cell volume is less than 12%, the animal has very severe anaemia, which can cause heart failure. Animals with very severe anaemia need a blood transfusion. Give 10 ml of packed red cells or whole blood per kg of body weight *slowly* over 3 hours. Do *not* give iron during the initial phase of treatment, as it can have toxic effects and may reduce resistance to infection.

CONGESTIVE HEART FAILURE

This is usually a complication of overhydration (especially when an IV infusion or standard ORS solution is given), very severe anaemia, blood or plasma transfusion, or giving a diet with a high sodium content. The first sign of heart failure is fast breathing. Later signs are respiratory distress, a rapid pulse, engorgement of the jugular vein, cold hands and feet, and cyanosis of the fingertips and under the tongue. Heart failure must be differentiated from respiratory infection and septic shock, which usually occur within 48 hours of admission, whereas heart failure usually occurs somewhat later. When heart failure is caused by fluid overload, the following measures should be taken:

- Stop *all* oral intake and IV fluids; the treatment of heart failure takes precedence over feeding the animal. No fluid should be given until the heart failure is improved, even if this takes 24–48 hours.
- Give a diuretic IV.2 The most appropriate choice is furosemide (1 mg/kg).

- Do not give digitalis unless the diagnosis of heart failure is unequivocal (jugular venous pressure is elevated) *and* the plasma potassium level is normal. In that case, 5µg/kg of body weight of digoxin may be given IV as a single dose, or orally, if the IV preparation is not available.

DERMATOSIS OF KWASHIORKOR

This is characterized by hypo- or hyperpigmentation, shedding of the skin in scales or sheets, and ulceration of the skin of the perineum, groin, limbs, behind the ears and armpits. There may be widespread weeping skin lesions which easily become infected.
Spontaneous resolution occurs as nutrition improves. Atrophy of the skin in the perineum can lead to severe dermatitis, especially if the animal has diarrhoea. In other affected areas, application of zinc and castor oil ointment, petroleum jelly or paraffin gauze dressings helps to relieve pain and prevent infection. The zinc supplement contained in the mineral mix is particularly important in these animals, as they are usually severely deficient.
Bathe the affected areas in 1% potassium permanganate solution for 10–15 minutes daily. This dries the lesions, helps to prevent loss of serum, and inhibits infection. Polyvidone iodine, 10% ointment, can also be used. It should be used sparingly, however, if the lesions are extensive, as there is significant systemic absorption. All animals with kwashiorkor-related dermatosis should receive systemic antibiotics.
There is no reported experience in malnourished animals of angiotensin-converting enzyme inhibitors or other drugs used to treat congestive heart failure. Diuretics should ***never*** be used to reduce oedema in malnourished animals.

REHABILITATION

The animal is deemed to have entered the rehabilitation phase *when his or her appetite has returned.* An animal which is being fed by NG tube is *not* considered ready to enter the rehabilitation phase.

Principles of Management

When *all* the criteria in the box below have been met (usually 2–3 weeks after arrival), treatment can concentrate on nutritional rehabilitation.

Criteria for Transfer to a Nutrition Rehabilitation Regime

- Eating well
- Mental state has improved: responds to stimuli, interested in surroundings
- Sits, crawls, stands or walks (depending on age)
- Normal temperature (36.5–37.5 °C for ape)
- No vomiting or diarrhoea
- No oedema
- Gaining weight: >5 g/kg of body weight per day for 3 successive days

NUTRITIONAL REHABILITATION

The most important determinant of the rate of recovery is the amount of energy consumed. However, at the start of the rehabilitation phase, the animal is still deficient in protein and various micronutrients, including potassium, magnesium, iron and zinc. These must also be given in increased amounts.

Feeding Chimps Under 12 Months

During rehabilitation, F-100 diet should be given every 4 hours, night and day. Transition to the rehabilitation phase involves increasing the amount of diet given at each feed by 10 ml (e.g. if the first feed is 60 ml, the second should be 70 ml, the third 80 ml, and so on) until the animal refuses to finish the feed. When a feed is not finished, the same amount should be offered at the next feed. If that feed is finished, the amount offered for the following feed should be increased by 10 ml. Continue this process until some food is left after most feeds. The amount being offered should then be dispensed for the animal at each feed on subsequent days. The amounts of each feed offered and taken should be recorded on a feeding chart and any food not taken should be discarded; *never* reuse it for the next feed.

During rehabilitation most **children** take between 150 and 220 kcal/kg (630–920 kJ/kg) per day (should be similar for non human primates). If intake is below 130 kcal or 540 kJ/kg per day, use this as an indicator for failing to respond. The attitude of those feeding the animal is crucial to success. Sufficient time must be spent with the animal to enable it to finish each feed. During the first few days of rehabilitation, animals with oedema may not gain weight, despite an adequate intake. This is because oedema fluid is being lost while tissue is being restored. Thus, progress in these individuals is seen as decreased oedema rather than rapid weight gain. If the animal is neither gaining weight nor showing decreased oedema, or if there is increasing oedema, it is failing to respond.

Feeding Chimps Over 12 Months to Adult

These animals can also be successfully treated with increasing quantities of F-100; it is not essential to use a different diet. For most older animals, however, it is appropriate to introduce solid food, especially for those who want a mixed diet. Most traditional mixed diets have a lower energy content than F-100. They are also relatively deficient in minerals, particularly potassium and magnesium, and contain substances which inhibit the absorption of zinc, copper and iron. Moreover, the diets are usually deficient in various vitamins. Thus, local foods should be fortified to increase their content of energy, minerals and vitamins. Oil should be added to increase the energy content, and the mineral and vitamin mixes used in F-100 should be added. Other ingredients, such as dried skimmed milk, may also be added to increase the protein and mineral content. The energy content of mixed diets should be *at least* 1 kcal or 4.2 kJ/g.

To avoid the effects of food substances which reduce the absorption of minerals, F-100 should be given between feeds of the mixed diet. For example,

if the mixed diet is given three times daily, F-100 should also be given three times daily, making six feeds a day.
At the beginning of rehabilitation, animals should be fed every 4 hours, day and night (six feeds per 24 hours). When they are growing well and are no longer at risk of developing hypothermia or hypoglycaemia, one of the night-time feeds can be omitted, making five feeds per 24 hours.

Folic Acid and Iron

Nearly all severely malnourished primates have anaemia and should be given supplementary folic acid and iron. They should also continue to receive the vitamin and mineral mixes in their food throughout rehabilitation. Iron should *never* be given during the initial phase of treatment, but must be given during the rehabilitation phase. It should only be given orally, *never* by injection. Animals with moderate or severe anaemia should be given elemental iron, 3 mg/kg per day in two divided doses, up to a maximum of 60mg daily, for 3 months (*7*). It is preferable to give iron supplements between meals using a liquid preparation. All animals must be given 5 mg of folic acid on day 1 and then 1mg per day thereafter. Folic acid is usually included in vitamin premixes (see appendix 2).

Assessing Progress

The animal should be weighed daily and the weight plotted on a graph. The usual weight gain is about 10–15 g/kg per day. An animal who does not gain *at least* 5 g/kg per day for 3 consecutive days is failing to respond to treatment. With high-energy feeding, most severely malnourished animals reach their target weight after 2–4 weeks.

Emotional and Physical Stimulation

Severely malnourished animals have delayed mental and behavioural development, which, if not treated, can become the most serious long-term result of malnutrition. Emotional and physical stimulation through play programmes that start during rehabilitation and continue after discharge can substantially reduce the risk of permanent mental retardation and emotional impairment.

Physical Activities

Physical activities promote the development of essential motor skills and may also enhance growth during rehabilitation. For those animals who are unable to move, passive limb movements and splashing in a warm bath are helpful. For other juveniles, normal play behaviour should be encouraged.

FAILURE TO RESPOND TO TREATMENT

General Principles

When the treatment guidelines in this manual are followed, a severely malnourished animal without complications should show definite signs of

improvement within a few days and should continue to improve thereafter. Failure to achieve initial improvement at the expected rate is termed *primary failure to respond*, whereas deterioration of the animal's condition, when a satisfactory response has been established, is termed *secondary failure to respond*. An animal who meets any of the criteria in Table 9 should be diagnosed as *failing to respond*. When this diagnosis is made it is essential that practices in the treatment unit are carefully reviewed and the animal is thoroughly re-evaluated. The objective is to identify the cause for failure to respond and to correct the problem by making specific changes to practices at the sanctuary or to the individual's treatment. Treatment should never be changed blindly; this is more likely to be harmful than to help the animal. The most frequent causes of failure to respond are listed in the box below.

Frequent causes of failure to respond

Problems with the treatment facility
- Insufficient or inadequately trained staff
- Inaccurate weighing machines
- Food prepared or given incorrectly

Problems of individual cases
- Insufficient food given
- Vitamin or mineral deficiency
- Malabsorption of nutrients
- Infections, especially diarrhoea, dysentery, otitis media, pneumonia, tuberculosis, urinary tract infection, malaria, intestinal helminthiasis
- Serious underlying disease

Criteria	Time after Admission
Primary failure to respond	
Failure to regain appetite	Day 4
Failure to start to lose oedema	Day 4
Oedema still present	Day 10
Failure to gain at least 5 g/kg of body weight per day	Day 10
Secondary failure to respond	
Failure to gain at least 5 g/kg of body weight per day for 3 successive days	During rehabilitation

Table 9. Criteria for failure to respond to treatment

Problems with the Treatment Facility

Type of Facility

The risk of cross infection is increased in a general area, it is more difficult to provide the necessary care and attention, and staff are less likely to have the essential skills and attitudes for management of malnourished animals.

Malnourished animals should be treated in a specially designated area. If essential food supplies or medications are not available, weighing scales do not work properly, diagnostic facilities or administrative procedures are inadequate, or there are insufficient trained staff, treatment failure and mortality will be high.

Inaccurate Weighing Machines

Weighing machines must be checked and adjusted daily following a standard procedure. Records of daily checks should be kept. Weighing machines used for preparing food or for measuring the ingredients of the mineral mix should be checked and adjusted weekly.

Problems with Preparing or Giving Food

Standard hygiene practices should be used when storing, preparing and handling food in the Sanctuary kitchen. Hands should be washed with soap after defecation and before food is handled. Persons with infections on their hands should not handle food. Each person involved in preparing food should be checked to ensure that they are following the correct procedures for weighing, measuring, mixing, cooking and storing the food. Observe the feeds being made; check that the recipes are correct and all ingredients are added. Ensure that sufficient time is allocated to feeding each animal and that there are enough staff, day and night, for this task. Assume that it takes about 15 minutes to feed each case and that food is given every 3 hours.

Problems with Individual Animals

Feeding

Is enough food being given?
Recalculate the food requirement for the animal. Ensure that the correct amount is being offered at the required times, and that the amount taken by the animal is measured and recorded accurately. Observe the measuring and giving of food. Check the calculation of the daily energy intake of the animal.
Are sufficient vitamins and minerals being given?

Nutrient deficiency can result from the increased requirements related to the synthesis of new tissue during rapid growth. When this happens, there is usually an initial period of rapid growth, after which growth slows or stops even though food intake is adequate. Deficiencies of potassium, magnesium, zinc, copper or iron may be responsible. Diets are often deficient in these minerals and commercial vitamin and mineral preparations do not provide them in sufficient amounts for severely malnourished animals. This problem can be avoided by ensuring that the mineral and vitamin mixes described in Appendix 2 are added to the animal's food every day.

Infection

Unrecognized infections are a frequent cause of failure to respond. Those most often overlooked include pneumonia, urinary tract infection, and tuberculosis.

Others include malaria, worms, and viral hepatitis infection. Animals who fail to respond to treatment should be investigated for infection as follows:

- Examine the animal carefully. Measure the animal's temperature, pulse rate and respiration rate every 3 hours. As already mentioned, infection in a malnourished animal often causes hypothermia.
- If possible, obtain a chest X-ray. Examine the urine for pus cells. Examine and culture the sputum or a tracheal aspirate for tubercle bacilli. Examine the stool for signs of blood, *Giardia* trophozoites or cysts and *Strongyloides stercoralis/fulliboeni* larvae, and culture for bacterial pathogens. Culture the blood and test for the presence of viral hepatitis B and malaria. Examine and culture the cerebrospinal fluid. Specific infections are discussed below.

Persistent diarrhoea

This is diarrhoea that occurs every day for at least 14 days. Weight loss is common. ReSoMal should be given to prevent or treat dehydration. If the stool contains visible blood, treat the animal with an oral antimicrobial that is effective against most local strains of *Shigella* (see treatment guidelines for dysentery below). If cysts or trophozoites of *Giardia* are found in the stool, treat the animal with metronidazole, 5 mg/kg orally three times daily for 5 days (or tinadazole once a day for three days). Blind antimicrobial therapy, however, is ineffective and should not be given. Every animal with persistent diarrhoea should be examined for non-intestinal infections, such as pneumonia, sepsis, urinary tract infection and otitis media. Antimicrobial treatment of these infections should follow standard guidelines. Antidiarrhoeal drugs should *never* be used. Such drugs are not effective in animals and some may be dangerous.

Dysentery

This is diarrhoea with visible blood in the stool. *Shigella* is the most frequent cause, especially of cases that are severe. Treatment is with an oral antibiotic to which most local strains of *Shigella* are sensitive. Unfortunately, the choice of antimicrobials for treatment of shigellosis has narrowed considerably in recent years as the prevalence of antimicrobial resistance has increased. Resistance to ampicillin and cotrimoxazole (sulfamethoxazole, trimethoprim), formerly the drugs of choice, is now widespread. Nevertheless, cotrimoxazole (25mg of sulfamethoxazole □□5mg of trimethoprim/kg orally twice daily for 5 days) and, in a few areas, ampicillin (25mg/kg four times daily for 5 days) may still be effective against most endemic strains. Nalidixic acid (15mg/kg four times daily for 5 days), which was formerly reserved for the treatment of resistant cases of shigellosis, is now the drug of choice in many areas. If there is no improvement (less blood in the stool or passage of fewer stools) after 2 days, the antibiotic should be changed to another to which local strains of *Shigella* are sensitive (see Appendix 4).

Accordingly, those sanctuaries located in areas where there is a high incidence of bloody diarrhoea in people should ensure that several antimicrobials known to be effective against most local strains of *Shigella* spp. are kept in stock.

Amoebiasis. Amoebiasis can cause dysentery, liver abscess and other systemic complications. Treatment for amoebiasis should be given when motile trophozoites of *Entamoeba histolytica* containing ingested erythrocytes are found in a fresh stool sample or when bloody diarrhoea continues after successive treatment with two antibiotics that are usually effective for *Shigella*. The finding of amoebic cysts in the stools is not sufficient for a diagnosis of amoebiasis. Treatment is with metronidazole oral suspension, 10 mg/kg three times daily for 5–10 days.
Giardiasis. In severely malnourished animals, treatment for giardiasis should be given when cysts or trophozoites of *Giardia* are seen in the stool. Treatment is with metronidazole, 5 mg/kg orally three times daily for 5 days.

Pneumonia

Pneumonia is manifested by fast breathing and, sometimes, chest indrawing. Cough, crackly breath sounds and abnormalities on chest X-ray are frequently absent. Animals with fast breathing should be diagnosed as having pneumonia and given an oral antimicrobial for 5 days. Cotrimoxazole (sulfamethoxazole, trimethoprim), ampicillin or amoxicillin is usually effective. **Children** with fast breathing and chest indrawing have been treated with benzylpenicillin, 50 000 IU/kg IM four times daily for at least 5 days, until they improved, and then with oral ampicillin or amoxicillin. Oxygen should also be given if the breathing rate is over 70 breaths per minute.

Urinary tract infections

Urinary tract infections occur frequently. Such infections are usually asymptomatic and are diagnosed using dip-stick tests or by finding large numbers of leukocytes on microscopic examination of fresh urine (at least 10 leukocytes per microscope field (X40 magnification)). Cotrimoxazole (25mg of sulfamethoxazole 5 mg of trimethoprim/kg twice daily for 5 days) is usually effective. Alternatively, ampicillin (25 mg/kg four times daily for 5 days) can be given.

Skin infections

Bacterial infections. These include pustules, impetigo, infected fissures (especially behind the ears) and indolent ulcers. Treatment should include washing the affected area with soap and water, and gently removing debris and crusts by soaking in warm saline or clean warm water. Dry the animal carefully and apply polyvidone iodine, 10% ointment, or chlorhexidine, 5% lotion, to the affected area. Widespread superficial and deep-seated infections should be treated with benzylpenicillin, 50000 IU/kg IM four times daily for at least 10 days. If abscesses are present, they should be drained surgically.

Tuberculosis

Tuberculosis is an important cause of failure to respond. The diagnosis is made by tuberculin screening and, if possible, chest X-ray and examination or culture of sputum or tracheal secretions.

Helminthiasis

Ascariasis, hookworm infection and trichuriasis. Whipworm infections can cause dysentery, anaemia and, occasionally, prolapse of the rectum. Hookworm infections can cause severe anaemia. Treatment of these infections should be delayed until the rehabilitation phase of treatment for severe malnutrition. Albendazole (400 mg in a single dose) and mebendazole (100 mg twice daily for 3 days or 500 mg in a single) are both effective. If these drugs are not available or the animal is under 2 years, hookworm can be treated with pyrantel (10 mg/kg in a single dose) and ascariasis with pyrantel or piperazine. Piperazine is also effective in whipworm infections.
Strongyloidiasis. Diagnosis of infection with *Strongyloides stercoralis* is made by detecting typical larvae in the faeces. In patients whose immune systems are depressed by disease, the larvae may become widely disseminated, giving rise to life-threatening pulmonary, cerebral and hepatic complications. Ivermectin should be given at 200-400 μg/kg in a single oral or subcutaneous dose.

Serious underlying disease

Malnutrition may result from unrecognized congenital abnormalities, inborn errors of metabolism, malignancies, immunological diseases and other diseases of the major organs. Examination of an animal who fails to respond to treatment should include a search for serious underlying disease. Any problem identified should be treated appropriately; however, the associated malnutrition should be managed according to the guidelines in this manual.

Learning from Failure

Accurate records should be kept of all animals who fail to respond to treatment and of all deaths. These should include, as a minimum, details of the animal's age, sex, date of arrival, weight-for-height (or length) on arrival, principal diagnoses, treatment and, where appropriate, date and time of death, and apparent cause of death. Periodic review of these records can help to identify areas where case management practices should be carefully examined and improved. For example, deaths that occur within the first 2 days are often due to hypoglycaemia, unrecognized or mismanaged septic shock, or other serious infection, whereas deaths that occur after day 2 are often due to heart failure. An increase in deaths occurring at night or at weekends suggests that monitoring and care of animals at those times should be reviewed and improved. The objective should be to achieve a case-fatality rate of <5%.

MALNUTRITION ADULT ANIMALS

Principles of Management

The physiological changes and principles of management of adults with severe malnutrition are the same as those in juveniles. In general, the guidelines for management of juveniles should be followed. There are, however, differences in the classification of malnutrition and the amount of food required.

History and Physical Examination

A thorough examination should be conducted to exclude conditions that give rise to secondary malnutrition. A careful dietary history should be taken. Blood sugar should be tested.

Initial Treatment

If possible, adults should be given the same formula feeds (with added minerals and vitamins) as juveniles. The initial goal of treatment is to prevent further loss of tissue. The amount of feed given per kg of body weight is much less than for juveniles and decreases with increasing age, reflecting the lower energy requirements of adults.
Adults are also susceptible to hypothermia and hypoglycaemia. The latter condition is managed as described for children. They should also be given systemic antibiotics and, except for pregnant animals, a single dose of 200 000 IU of vitamin A orally.

APPENDIX 1 PHYSIOLOGICAL BASIS FOR TREATMENT OF SEVERE MALNUTRITION

Affected organ or system	Effects	Treatment
Cardiovascular System	Cardiac output and stroke volume are reduced Infusion of saline may cause an increase in venous pressure Any increase in blood volume can easily produce acute heart failure; any decrease will further compromise tissue perfusion Blood pressure is low Renal perfusion and circulation time are reduced Plasma volume is usually normal and red cell volume is reduced	If the child appears dehydrated, give ReSoMal or F-75 diet Don't give fluids intravenously unless the child is in shock Restrict blood transfusion to 10ml/kg and give a diuretic
Liver	Synthesis of all proteins is reduced Abnormal metabolites of amino acids are produced Capacity of liver to take up, metabolize and excrete toxins is severely reduced Energy production from substrates such as galactose and fructose is much slower than normal Gluconeogenesis is reduced, which increases the risk of hypoglycaemia during infection Bile secretion is reduced	Do not give the child large meals Ensure that the amount of protein given does not exceed the metabolic capacity of the liver, but is sufficient to support synthesis of proteins (1–2 g/kg per day) Reduce the dosage of drugs that depend on hepatic disposal or are hepatotoxic Ensure that sufficient carbohydrate is given to avoid the need for gluconeogenesis Do not give iron supplements, which may be dangerous because transferring levels are reduced
Genitourinary System	Glomerular filtration is reduced Capacity of kidney to excrete excess acid or a water load is greatly reduced Urinary phosphate output is low Sodium excretion is reduced Urinary tract infection is common	Prevent further tissue breakdown by treating any infections and providing adequate energy (80–100kcalth or 336–420 kJ/kg per day) Do not give the child more protein than is required to maintain tissues Ensure that high-quality proteins are given, with balanced amino acids Avoid nutrients that give an acid load, such as magnesium chloride Restrict dietary sodium. Ensure that water intake is sufficient but not excessive
Gastrointestinal System	Production of gastric acid is reduced Intestinal motility is reduced Pancreas is atrophied and production of digestive enzymes is reduced Small intestinal mucosa is atrophied; secretion of digestive enzymes is reduced Absorption of nutrients is reduced when large amounts of food are eaten	Give the animal small, frequent feeds If absorption is poor, increase the frequency and reduce the size of each feed If there is malabsorption of fat, treatment with pancreatic enzymes may be useful
Affected organ or system	**Effects**	**Treatment**
Immune system	All aspects of immunity are diminished Lymph glands, tonsils and the thymus are atrophied Cell-mediated (T-cell) immunity is severely depressed	Treat all children with broad-spectrum antimicrobials Because of the risk of transmission of infection, ensure that newly arrived individuals are kept apart from others who are recovering from infection

	IgA levels in secretions are reduced Complement components are low Phagocytes do not kill ingested bacteria efficiently Tissue damage does not result in inflammation or migration of white cells to the affected area Acute phase immune response is diminished Typical signs of infection, such as an increased white cell count and fever, are frequently absent Hypoglycaemia and hypothermia are both signs of severe infection and are usually associated with septic shock	
Endocrine system	Insulin levels are reduced and the animal has glucose intolerance	Give small, frequent feeds
	Insulin growth factor 1 (IGF-1) levels are reduced, although growth hormone levels are increased Cortisol levels are usually increased	Do not give steroids
Circulatory system	Basic metabolic rate is reduced by about 30%	Keep the animal warm to prevent hypothermia; keep the temperature of the living environment at 25–30 ^{0}C
	Energy expenditure due to activity is very low	
	Both heat generation and heat loss are impaired; the animal becomes hypothermic in a cold environment and hyperthermic in a hot environment	If an animal has a fever, cool the animal by sponging with tepid (not cold) water (*never* alcohol rubs)
Cellular function	Sodium pump activity is reduced and cell membranes are more permeable than normal, which leads to an increase in intracellular sodium and a decrease in intracellular potassium and magnesium Protein synthesis is reduced	Give large doses of potassium and magnesium to all patients Restrict sodium intake
Skin, muscles and glands	The skin and subcutaneous fat are atrophied, which leads to loose folds of skin Many signs of dehydration are unreliable; eyes may be sunken because of loss of subcutaneous fat in the orbit Many glands, including the sweat, tear and salivary glands, are atrophied; the child has dryness of the mouth and eyes and sweat production is reduced Respiratory muscles are easily fatigued; the animal is lacking in energy	Rehydrate the animal with ReSoMal or F-75 diet

APPENDIX 2 COMPOSITION OF MINERAL AND VITAMIN MIXES

COMPOSITION OF MINERAL MIX SOLUTION

Substance Amount
Potassium chloride 89.5 g
Tripotassium citrate 32.4 g
Magnesium chloride ($MgCl_2 \cdot 6H_2O$) 30.5 g
Zinc acetate 3.3 g
Copper sulfate 0.56 g
Sodium selenatea 10mg
Potassium iodide 5mg
Water to make 1000 ml
a If it is not possible to weigh very small amounts accurately, this substance may be omitted.
The above solution can be stored at room temperature. It is added to ReSoMal or liquid feed at a concentration of 20 ml/litre.

COMPOSITION OF VITAMIN MIX

Vitamin Amount per litre of liquid diet Water-soluble:
Thiamine (vitamin B1) 0.7mg
Riboflavin (vitamin B2) 2.0mg
Nicotinic acid 10mg
Pyridoxine (vitamin B6) 0.7mg
Cyanocobalamin (vitamin B12) 1µg
Folic acid 0.35mg
Ascorbic acid (vitamin C) 100mg
Pantothenic acid (vitamin B5) 3mg
Biotin 0.1mg
Fat-soluble:
Retinol (vitamin A) 1.5mg
Calciferol (vitamin D) 30µg
ß-Tocopherol (vitamin E) 22mg
Vitamin K 40µg

APPENDIX 3 DESIRABLE DAILY NUTRIENT INTAKE DURING INITIAL PHASE OF TREATMENT

NUTRIENT AMOUNT PER KG OF BODY WEIGHT
Water 120–140 ml
Energy 100 kcal (420 kJ)
Protein 1–2 g
Electrolytes:
Sodium 1.0 mmol (23 mg)
Potassium 4.0 mmol (160 mg)
Magnesium 0.6 mmol (10 mg)
Phosphorus 2.0 mmol (60 mg)
Calcium 2.0 mmol (80 mg)
Trace minerals:
Zinc 30µmol (2.0mg)
Copper 4.5µmol (0.3mg)
Selenium 60 nmol (4.7µg)
Iodine 0.1µmol (12µg)
Water-soluble vitamins:
Thiamine (vitamin B1) 70µg
Riboflavin (vitamin B2) 0.2mg
Nicotinic acid 1mg
Pyridoxine (vitamin B6) 70µg
Cyanocobalamin (vitamin B12) 0.1mg
Folic acid 0.1mg
Ascorbic acid (vitamin C) 10µg
Pantothenic acid (vitamin B5) 0.3mg
Biotin 10µg
Fat-soluble vitamins:
Retinol (vitamin A) 0.15mg
Calciferol (vitamin D) 3µg
ß -Tocopherol (vitamin E) 2.2mg
Vitamin K 4µg
(a) Value refers to the maximum recommended daily intake.

APPENDIX 4 DRUG DOSAGES FOR TREATMENT OF INFECTIONS

Antimicrobials

Amoxicillin 15 mg/kg orally every 8 hours tablet, 250 mg (anhydrous) syrup, 250 mg/5 ml

Ampicillin 25 mg/kg orally every 6 hours tablet, 250mg 50 mg/kg IM or IV every 6 hours powder for injection, 500 mg (as sodium salt) in vial, mixed with 2.5 ml of sterile water

Benzylpenicillin 50 000 IU/kg IM or IV every 6 hours powder for injection, 600 mg (= 1 million IU) (as
sodium or potassium salt), mixed with 1.6 ml of sterile water (for IM injection) or 10 ml of sterile
water (for IV injection)

Chloramphenicol 25 mg/kg IM or IV every 6 hours powder for injection, 1 g (as sodium succinate) in
(for meningitis only) or every 8 hours vial, mixed with 3.2 ml of sterile water (for IM (for other conditions) injection) or 9.2 ml of sterile water (for IV injection)

Cotrimoxazole 25 mg of sulfamethoxazole, 5mg paediatric tablet, 100 mg of sulfamethoxazole of trimethoprim/kg orally every 20 mg of trimethoprim 12 hours syrup, 200 mg of sulfamethoxazole 40 mg of trimethoprim/5 ml

Gentamicin 7.5 mg/kg IM or IV once daily injection, 10 mg (as sulfate)/ml in 1-ml vial injection, 20 mg, 40 mg, 80 mg (as sulfate)/ml in 2-ml vial

Metronidazole Amoebiasis: 10 mg/kg orally every tablet, 200 mg, 400mg 8 hours for 5–10 days

Giardiasis: 5 mg/kg orally every 8 hours for 5 days

Nalidixic acid 15 mg/kg orally every 6 hours tablet, 250mg IM: intramuscularly, IV: intravenously.

Anthelmintics

Ivermectin 200µg/kg in a single dose Strongyloidiasis

Levamisole 2.5 mg/kg in a single dose Ascariasis, hookworm infections and trichuriasis

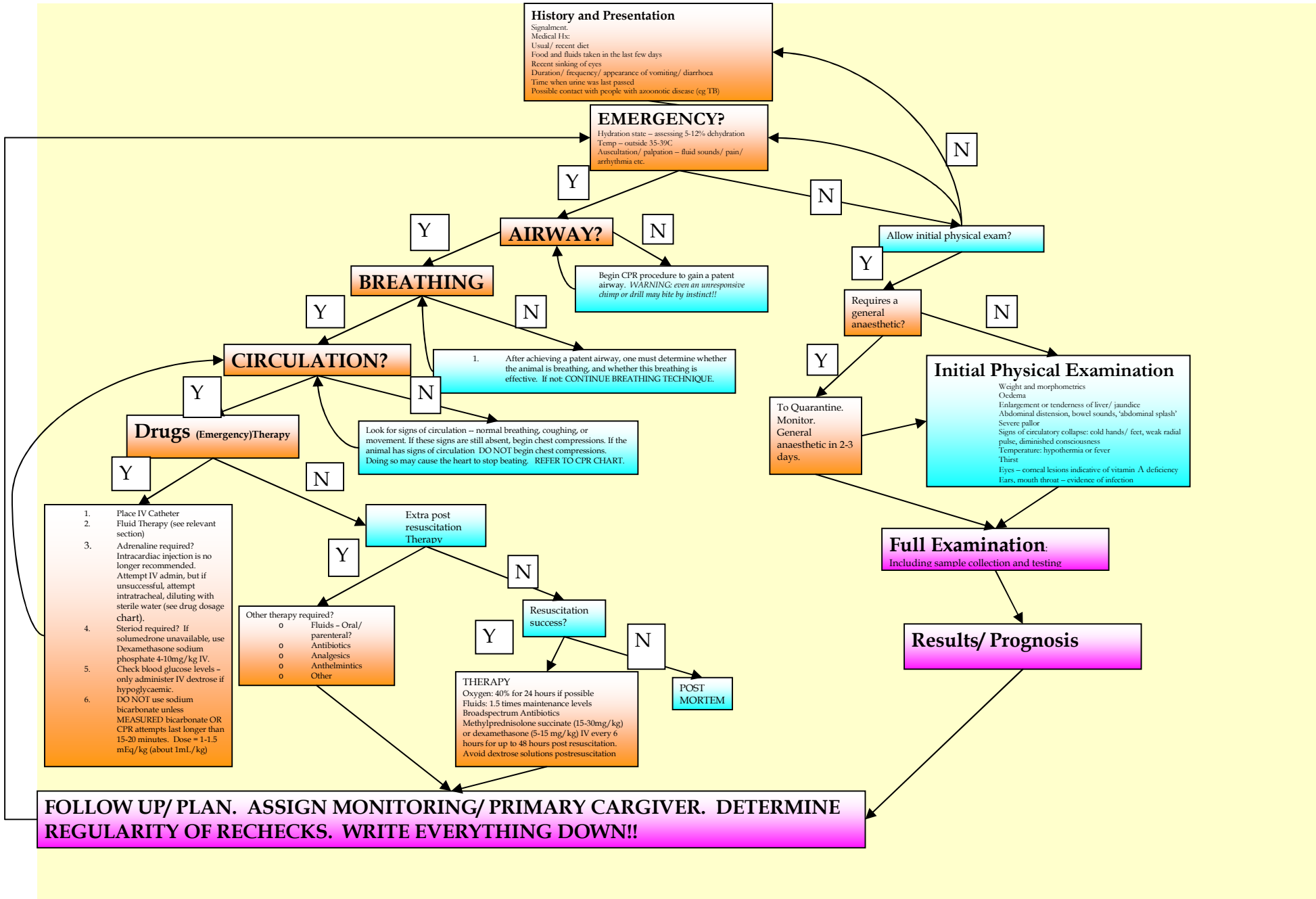
Mebendazole 100 mg once daily for 3 days Ascariasis, hookworm infections and trichuriasis

Pyrantel 10 mg/kg in a single dose Ascariasis and hookworm infections

3.8 EMERGENCY MEDICINE

S.Unwin

Use this page for your planning your own emergency protocols


History and Presentation
Signalment.
Medical Hx:
Usual/ recent diet
Food and fluids taken in the last few days
Recent sinking of eyes
Duration/ frequency/ appearance of vomiting/ diarrhoea
Time when urine was last passed
Possible contact with people with azoonotic disease (eg TB)
EMERGENCY?
Hydration state – assessing 5-12% dehydration
Temp – outside 35-39C
Auscultation/ palpation – fluid sounds/ pain/ arrhythmia etc.
Y
N
AIRWAY?
Y
N
Begin CPR procedure to gain a patent airway. WARNING: even an unresponsive chimp or drill may bite by instinct!!
BREATHING
Y
N
1. After achieving a patent airway, one must determine whether the animal is breathing, and whether this breathing is effective. If not: CONTINUE BREATHING TECHNIQUE.
CIRCULATION?
Y
N
Look for signs of circulation – normal breathing, coughing, or movement. If these signs are still absent, begin chest compressions. If the animal has signs of circulation DO NOT begin chest compressions. Doing so may cause the heart to stop beating. REFER TO CPR CHART.
Drugs (Emergency)Therapy
Y
N
1. Place IV Catheter
2. Fluid Therapy (see relevant section)
3. Adrenaline required? Intracardiac injection is no longer recommended. Attempt IV admin, but if unsuccessful, attempt intratracheal, diluting with sterile water (see drug dosage chart).
4. Steriod required? If solumedrone unavailable, use Dexamethasone sodium phosphate 4-10mg/kg IV.
5. Check blood glucose levels – only administer IV dextrose if hypoglycaemic.
6. DO NOT use sodium bicarbonate unless MEASURED bicarbonate OR CPR attempts last longer than 15-20 minutes. Dose = 1-1.5 mEq/kg (about 1mL/kg)
Extra post resuscitation Therapy
Y
N
Other therapy required?
o Fluids – Oral/ parenteral?
o Antibiotics
o Analgesics
o Anthelmintics
o Other
Resuscitation success?
Y
N
THERAPY
Oxygen: 40% for 24 hours if possible
Fluids: 1.5 times maintenance levels
Broadspectrum Antibiotics
Methylprednisolone succinate (15-30mg/kg) or dexamethasone (5-15 mg/kg) IV every 6 hours for up to 48 hours post resuscitation.
Avoid dextrose solutions postresuscitation
POST MORTEM
N
Allow initial physical exam?
Y
Requires a general anaesthetic?
Y
N
To Quarantine. Monitor. General anaesthetic in 2-3 days.
Initial Physical Examination
Weight and morphometrics
Oedema
Enlargement or tenderness of liver/ jaundice
Abdominal distension, bowel sounds, 'abdominal splash'
Severe pallor
Signs of circulatory collapse: cold hands/ feet, weak radial pulse, diminished consciousness
Temperature: hypothermia or fever
Thirst
Eyes – corneal lesions indicative of vitamin A deficiency
Ears, mouth throat – evidence of infection
Full Examination:
Including sample collection and testing
Results/ Prognosis
FOLLOW UP/ PLAN. ASSIGN MONITORING/ PRIMARY CARGIVER. DETERMINE REGULARITY OF RECHECKS. WRITE EVERYTHING DOWN!!

Emergency medicine is a constantly changing field. These notes are designed to form the basis of emergency treatment at your facility, but they are NOT COMPLETE. The information presented here by necessity is a mixture of domestic and zoo animal protocols, and human therapy recommendations.

During an emergency it is very important that you remain calm. Animals can sense your unease, but cannot understand what is happening and you cannot verbally tell them. Your body language is very important. Be calm, yet deliberate in your actions.

It is recommended you read the companion notes on primate fluid therapy. These notes contain:

- Suggested emergency box contents
- Emergency medication dose rates (also refer to the Formulary)
- Cardiopulmonary resuscitation- based on canine and human (adult and infant) protocols
- Hyperthermia-induced cardiac arrest in monkeys: limited efficacy of standard CPR - published paper abstract
- IV access technique – web published veterinary notes (canine)
- Pneumothorax
- Severe wounds
- Haemorrhagic gastroenteritis
- Hypoglycaemia
- Seizures
- Appendix – Management of the trauma patient integrated approach – Web published veterinary notes

EMERGENCY DRUG BOX

This is in addition to an anaesthetic box – both of which are on hand for all procedures

Anicath I/V catheters – size 14g, 18g, 22g, 26g (x1 of each)

Catheter stoppers with injection port x 2

Surgical spirit

Cotton wool

Hepflush (Heparinised saline for catheters)

Elastoplast 2.5cm

Tissue glue

Monoject needles – pink (18g) x5
Yellow (20g) x 5

Syringes – 1ml, 2ml, 5ml, 10ml, 20ml – at least 2 of each size

(minimum drug levels)
Article II. Diazepam 5mg/ml x 2ml x 10 vials
Adrenaline 1:100 1mg/ml x1ml x 20 vials
Dopram v injection 25ml x 1
Atropine (atrocare) 25ml x 1
Frusemide 5% 10ml x1
Dexadreson 50ml x 1
Solumed 8ml x 6 vials
Water for injection 100ml x 1

Dose chart (see table 1)

Table 1. EMERGENCY DRUG DOSES (Canine, but can be used for primates as well) for INTRAVENOUS injection (or double dose and dilute to 5-10mL for intratracheal deposition).

Drug	Trade Name	Dose	Reason	5Kg	10Kg	25Kg	50Kg
ADRENALINE 1:1000 = 1mg/mL	Adrenaline – can dilute	0.1mg/kg (0.01-0.2mg/kg) 0.01mg/kg	CARDIAC ARREST – give IV asystole/ fibrillation	0.5mL	1mL	2.5mL	5mL
			ANAPHYLAXIS – give IM	0.05mL	0.1mL	0.25mL	0.5mL
ATROPINE 0.6mg/mL	Atropine	0.04mg/kg	CARDIAC ARREST, asystole, vagally induced arrythmias	0.33mL	0.66mL	1.66mL	3.33mL
GLYCOPYRROLATE 200ug/mL	Robinul	(5-) 10ug/kg	CARDIAC ARREST asystole, vagally induced arrythmias	0.25mL	0.5mL	1.25mL	2.5mL
LIDOCAINE 20mg/mL	Xylocaine 2% Lignocaine 2%	1-5mg/kg slowly then infusion at 25-100ug/kg/min	Ventricular arrhythmias/ ventricular fibrillation	0.25-1.25mL	0.5-2.5mL	1.25-6.25mL	2.5-12.5mL
PROPRANOLOL 1mg/mL	Inderal can dilute in saline	0.02-0.08mg/kg slowly	ARRHYTHMIAS supraventricular tachycardia	0.1-0.4mL	0.2-0.8mL	0.5-2mL	1-4mL
VERAPAMIL 2.5mg/kg	Securon	0.05mg/kg slowly	Supraventricular tachyarrhythmias	0.1mL	0.2mL	0.5mL	1mL
ISOPRENALINE 1mg/mL	Saventrine	0.04-0.1ug/kg/min infuse to effect	BRADICARDIA/ AV BLOCK	To effect	To effect	To effect	To effect
FRUSEMIDE 5% 50mg/mL	Lasix 5%	(1-) 2 (-4) mg/kg	OEDEMA/ DIURESIS	0.2mL	0.4mL	1mL	2mL
DEXTROSE 5-50% 50-500mg/mL	Dextrose/ glucose	0.25-0.5g/kg slowly	HYPOGLYCAEMIA not by peripheral vein if >10% solution				
DOXAPRAM 20mg/mL	Dopram V	(1-) 5 (-10) mg/kg	RESPIRATORY STIMULANT	1.25mL	2.5mL	6.25mL	12.5mL
METHYL-PRED SUCCINATE 125 or 500mg	Solu-medrone	30mg/kg	SHOCK/ ALLERGY/ CEREBRAL OR SPINAL OEDEMA				
DIAZAPAM 5mg/mL	Diazapam/ vallium/ pamlin	0.5mg/kg	SEIZURES	0.5mL	1mL	2.5mL	5mL

CARDIO PULMONARY RESUSCITATION – A B C D E

If an animal is breathing, its heart MUST be beating also.

A = Airway

The first step in animal CPR, after determining non-responsiveness, is to obtain a patent airway. You should not continue on, until this step has been achieved.

1. Carefully pull the tongue out of the animal's mouth
   WARNING: even an unresponsive chimp or drill may bite by instinct!!
2. Make sure that the neck is reasonably straight; try to bring the head in-line with the neck.
   WARNING: Do not hyperextend in cases where neck trauma exists.
3. Attempt 2 rescue breaths, by closing the mouth, and performing **mouth-to-nose** ventilations. If they go in with no problems continue to **B**-Breathing.
4. Reposition the neck and try step 3 again.
5. Visibly inspect the airway by looking into the mouth, and down the throat for foreign objects occluding the airway. Unlike human-CPR, rescuers may reach into the airway and remove foreign objects that are visible.
6. Intubate – or -
7. **Procedure of performing an emergency tracheotomy (Figure 1)**

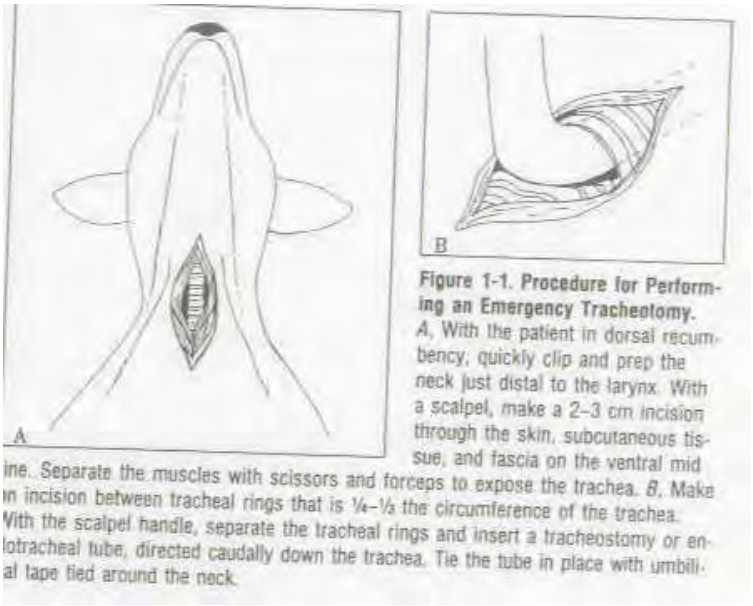


Figure 1-1. Procedure for Performing an Emergency Tracheotomy. *A*, With the patient in dorsal recumbency, quickly clip and prep the neck just distal to the larynx. With a scalpel, make a 2–3 cm incision through the skin, subcutaneous tissue, and fascia on the ventral midline. Separate the muscles with scissors and forceps to expose the trachea. *B*, Make an incision between tracheal rings that is ¼–⅓ the circumference of the trachea. With the scalpel handle, separate the tracheal rings and insert a tracheostomy or endotracheal tube, directed caudally down the trachea. Tie the tube in place with umbilical tape tied around the neck.

Figure 1. Procedure for perfoming an emergency tracheotomy. After Plunkett 1993 Emergency Procedures for the Small Animal Veterinarian. WB Saunders).

B = Breathing

After intubation, ventilatory assistance is the same as humans. Bag-Valve resuscitators are the normal instrument of choice (if you can get one donated),

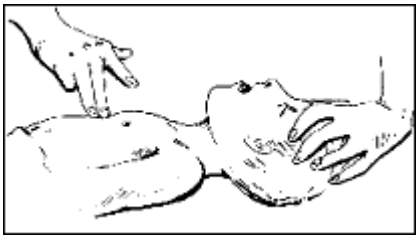
however a demand-valve power-resuscitator should work fine. Some animals will not tolerate a mask of any kind, so use high-flow blow-by on breathing animals in respiratory distress.

1. After achieving a patent airway, one must determine whether the animal is breathing, and whether this breathing is effective. If not:
2. Carefully pull the tongue out of the animal's mouth and check colour.
3. Make sure that the neck is reasonably straight; try to bring the head in-line with the neck. 100% oxygen administration, OR -
4. Ventilate the animal by closing the mouth, and performing mouth-to-nose ventilations. If they do not go in with ease go to A-Airway
5. Ventilate at 20-30 breaths per minute. Note that if supplemental Oxygen is available, and the animal is breathing on its own, use a high-flow blowby.
6. Proceed to C-Circulation, while continuing respiratory support as necessary

C = Circulation

This is the final step of CPR and should only be initiated after the airway and breathing steps have been completed. Make sure that there are no major (pooling/spurting blood) points of bleeding. Control as necessary. If a spinal injury is suspected, DO NOT tilt the head back when attempting to open the airway. Instead, place your fingers on the jaw on each side of the head. Lift the jaw forward. This keeps the head and neck from moving. Look for signs of circulation -- normal breathing, coughing, or movement. If these signs are still absent, begin chest compressions. If the animal has signs of circulation DO NOT begin chest compressions. Doing so may cause the heart to stop beating.

For guenons and mangabeys, and babies of other species, including the apes, the human infant protocol can be adapted:
1) Make sure animal is on a hard surface lying on their back.
2) Landmark – Create an imaginary line between the animal's nipples. Take your index finger and put it on the centre of the line. On the same hand use your middle finger and your ring finger and place them beside your index finger which is on the imaginary line. These two fingers will be what you use to perform the chest compressions. (See figure 2)



Figur 2. Method of chest compression in a baby/ small primate during CPR

3) Perform chest compressions with the two fingers on the animal's chest. Use your free hand to secure the infants head and to tilt the head back to open the airway. 20 cycles of 5 chest compressions to 1 breathe (puff). Make sure the compressions are about 2 compressions / second. It might seem fast but it's needed for proper circulation. Make sure each compression is 0.5 to 1 inches in depth.

For larger monkeys and chimpanzees, the following can apply:

1. Make sure animal is on a hard surface lying on their back.
2. Simultaneous chest compressions and ventilation every 2-3 compressions
3. 80-120 chest compressions per minute
4. Compress chest in a 'cough like manner' causing approximately 30% displacement of the chest wall
5. Application of a counter pressure device (bandages etc) to the caudal half of the patient may be of benefit

For gorillas, the adult human protocol can apply:

1. Make sure animal is on a hard surface lying on their back.
2. Perform chest compressions:
3. Place the heel of one hand on the breastbone -- right between the nipples.
4. Place the heel of your other hand on top of the first hand.
5. Position your body directly over your hands. Your shoulders should be in line with your hands. DO NOT lean back or forward. As you gaze down, you should be looking directly down on your hands.
6. Give 15 chest compressions. Each time, press down about 2 inches into the chest. These compressions should be FAST with no pausing. Count the 15 compressions quickly: "a, b, c, d, e, f, g, h, i, j, k, l, m, n, off."
7. Give the animal 2 slow, full breaths. The chest should rise.
8. Continue cycles of 15 chest compressions followed by 2 slow, full breaths.
9. After about 1 minute (four cycles of 15 compressions and 2 breaths), re-check for signs of circulation.
10. Repeat steps 11 and 12 until the animal recovers –then place in the recovery position.

D = Drug Therapy

1. Place IV Catheter
    - <6.5kg, 20 GA needle
    - 6.5-10 kg, 18 GA needle
    - 11-15 kg, 16 GA needle
    - 16-21 kg, 14 GA needle
    - >21 kg, 2 or more 14 GA needles.
2. Fluid Therapy (see relevant section)
3. Adrenaline required? Intracardiac injection is no longer recommended. Attempt IV admin, but if unsuccessful, attempt intratracheal, diluting with sterile water (see drug dosage chart).
4. Steroid required? If solumedrone unavailable, use Dexamethasone sodium phosphate 4-10mg/kg IV.
5. Check blood glucose levels – only administer IV dextrose if hypoglycaemic.
6. DO NOT use sodium bicarbonate unless MEASURED bicarbonate OR CPR attempts last longer than 15-20 minutes. Dose = 1-1.5 mEq/kg (about 1mL/kg)

E = Extra – post-resuscitation therapy

a. Oxygen: 40% for 24 hours if possible
b. Fluids: 1.5 times maintenance levels (see fluid therapy notes)
c. Antibiotics – administer broad spectrum antibiotics
d. Methylprednisolone succinate (15-30mg/kg) or dexamethasone (5-15 mg/kg) IV every 6 hours for up to 48 hours post resuscitation
e. Avoid dextrose solutions post resuscitation as they seem to affect patients' neurologic status adversely.

Hyperthermia-induced cardiac arrest in monkeys: limited efficacy of standard CPR
Eshel G, Satar P, Radovsky A, Stezoski SW
Aviat Space Environ Med 1997; 68:415-20
Abstract
Background: Successful resuscitation from heatstroke cardiopulmonary arrest has been only partially explored and the data covering the post resuscitation pathophysiology leading to secondary arrest is, in most cases, insufficient. **Hypothesis:** Following heatstroke-cardiopulmonary arrest, successful resuscitation may be achieved by standard CPR with surface cooling and administration of glucose. We ponder the sequence of early circulatory responses and the pathophysiological changes following successful resuscitation. **Methods:** We exposed 12 pigtail monkeys to total- body hyperthermia (cerebral T 42°C) until cardiac arrest ensued. Standard external CPR with surface cooling and glucose 5% IV were administered for up to 30 min. Control group A (n = 6) was compared with experimental group B (n = 6), which received additional steroid, glucagon and hypertonic glucose during CPR attempts. **Results:** No significant differences were found between the outcome of the two groups. The 30-min CPR attempt succeeded in restoration of spontaneous circulation (ROSC) in 8/12 monkeys - 5 animals from group A and 3 in group B. The animals in whom resuscitation was unsuccessful had significantly prolonged periods of rectal temperature exceeding 42.5°C ($p < 0.05$), and significantly higher rectal temperatures at the end of 30 min of CPR and cooling ($p < 0.05$). All the resuscitated animals later rearrested at 158 ± 68 (95-228) min after ROSC; pulmonary edema occurred in 6/8 animals. **Conclusions:** We conclude that experimentally- induced heatstroke can be transiently reversed by standard resuscitative procedures, but is followed by a delayed, irreversible, secondary shock state, which could not be prevented by the treatment we employed. We were, however, able to document in detail the pathophysiologic processes involved in the resuscitation, and **the irreversible shock one sees after "successful" CPR.**

Pneumothorax

Many of the protocols in this section are based on Plunkett (1993) – Emergency procedures for the small animal veterinarian.

Diagnosis:
May or may not have a history of trauma. Dyspnoea, open-mouthed breathing, dullness on percussion, crepitation, cyanosis, diminished lung sounds, muffled heart sounds, hyperresonance with chest percussion.
Prognosis:
Guarded

Treatment:
A closed chest pneumothorax is often tolerated better by animals than by humans, however it is still an EMERGENCY SITUATION. Here is the technique for reducing a tension pneumothorax which must be done IMMEDIATELY:

a. Lay the animal lateral with the pneumothorax up
b. Find the 8th intercostal space
c. Using an 18 gauge needle, insert the needle on the caudal (inferior) margin of the 8th intercostal space at the dorsal third of the chest wall
d. Using a stop-cock or other venting technique, reduce the pleural tension

The pneumothorax is now 'open' and can be treated as followed

a. Oxygen administration
b. Bilateral thoracocentesis, possible chest tube placement (see below). Use sterile technique (clip and scrub site, wear sterile gloves, use sterile equipment). Depending on the degree of distress and the volume of air removed from the chest, a chest drain usually is not placed unless thoracocentesis has been performed twice.
c. Stabilization for shock/ minimise stress
d. Place an IV catheter – measure PCV and TP.
e. Treat the cause of the pneumothorax – IV fluids, corticosteroids for shock etc.
f. Administer antibiotics
g. If possible, keep the animal separated for at least 3-4 days to monitor.

Chest Tube Placement. Use of a chest drain will depend greatly on the ability of the animal to remove it and the increased possibility of infection. I have included tube placement here for completeness, but this would usually be an unrealistic option in primate species.

a. Clip, scrub, prep the seventh intercostal space on animal's side. Marcaine or lidocaine nerve block
b. Pull the skin forward, incise skin with scalpel blade (approximately 2cm long incision).
c. Make a small hole in the intercostals muscles.
d. Advance the chest tube (DO NOT USE A FOLEY CATHETER) through the hole into the chest with curved haemostats.
e. Suture the chest tube to intercostals muscles and skin.

f. Place a purse string suture in the skin around the placement site to improve security.
g. Apply antibiotic dressing (e.g. Betadine) and occlusive dressing.

Severe wounds (inc. wound dehiscence)

Deep wounds in primates are often left, and the animal placed on antibiotics – these usually heal well. The animal must however be closely monitored for signs of septicaemia and other complications. Lacerations on sexual swellings should be left alone until swelling reduces if closure is deemed appropriate. Lacerations on other parts of the body should be closed when the wound is still clean, or allowed to heal by second intention, depending on whether it is felt that sutures / tissue glue etc will cause the animal to pick at the wound. All sutures should be buried (intradermal).

Prognosis:

Excellent unless internal injuries, severe haemorrhage, or other injuries are present.

Treatment:

a. Control haemorrhage with pressure bandages if necessary.
b. Give antibiotic injection as soon as possible – for deep and dirty wounds, should be on antibiotics for 5-10 days.
c. Carefully assess the trauma patient before administering anaesthesia. Treat shock and life-threatening problems first
d. IF POSSIBLE – take radiographs – inject contrast media into bite wounds to follow their tracks and determine their depth.
e. Clip, scrub, flush, prep wounds. For deep contaminated wounds, consider using a dilute metronidazole (flagyl) or crystapen (penicillin) solution as the flush solution, otherwise flush with sterile saline or lactated ringers solution.
f. Explore wounds thoroughly, especially those over the chest and abdomen. If a wound perforates into the chest, then perform thoracocentesis.
g. Debride wounds thoroughly. Flush with large amounts of sterile saline/ LRS.

(Near) drowning

Diagnosis: By physical exam – Moist cough, cyanosis, dyspnoea, moist bronchovesicular sounds, epistaxis.

Prognosis: Guarded to poor.

Treatment:

a. Administer oxygen if possible. To reduce pulmonary oedema, if you have access to a nebuliser, administer the oxygen through the nebuliser filled with a 1:2 mixture of 100-proof vodka (or similar): sterile water.
b. Place an IV catheter
c. Furosemide IM, IV or PO (see table for doses) – diuretic.
d. Bronchodilaters:
e. Aminophylline 2-8mg/kg SLOW IV, IM or PO q8-12h.
f. Albuterol 0.02-0.05mg/kg PO q8h

g. Terbutaline 0.01mg/kg SC q4h.
h. Dexamethasone sodium phosphate 4-8mg/kg IV
i. Administer broad spectrum systemic antibiotics
j. Severe nonresponsive dyspnoea may require sedation and positive pressure ventilation, extremely poor prognosis
k. Nitroglycerin ointment and ½ inch applied to a clipped area on the thorax or abdomen q4-6h, especially for pulmonary oedema (venous dilator).

Haemorrhagic gastroenteritis

An easy way in an animal with severe diarrhoea, to check for occult blood, is to 'diff quik' a smear and check for blood cells. If these are present, this indicates that there is a breakdown of the gastrointestinal lining, and antibiotics may be indicated. If at all possible, diagnose the cause of the diarrhoea. If obvious haemorrhage (dysentery), treat as follows:

a. LRS 30-60mL mL/kg until the PCV drops below 50% - see fluid therapy notes for use of oral fluids
b. Antimicrobial therapy (penicillins) – IF INDICATED
c. Corticosteroids (2-4mg/kg dexamethasone sodium phosphate IV or 5-10mg/kg prednisolone succinate IV) if in shock – and only within 2 hours on onset (their use remains controversial).
d. Salicylates with or without pectin (e.g. Pepto-bismol), if no vomiting.
e. Nothing orally for 12-24 hours. Start on oral liquids after 12-24 hours, gradually introducing bland food (such as rice base/ bland baby food), then gradually switch back to regular diet.

Hypoglycaemia (covered in malnutrition notes also)

Hypoglycaemia is a serious, immediate life threat, and needs to be treated aggressively. As in humans, the results can be startling in how rapid a patient can recover (if at all) from severe hypoglycaemia.

a. Start LRS with D5 (LRS with 5% dextrose) IV Drip
b. Bolus 2-4ml/kg D50 (50% dextrose in water) or 20ml/kg D10 (10% dextrose in water)
c. Reassess the blood glucose levels if possible

Seizures

Although it is rare for an animal to require emergency care due to seizures, their treatment is the same as in humans: Mostly supportive. If possible, place a pad under the head to prevent trauma, and assure that there is no hazard from the environment to the patient. Do not stick ANYTHING in an animals mouth. If the animal sustains the seizure state for more than 5 minutes, then treat as *Status Epilepticus*. The 2 main drugs used in Veterinary medicine for the cessation of seizures is Diazepam (Valium) and Phenobarbital.

- Diazepam 1mg/kg (can work IM, but very slow action)
  Note: you can also administer rectally, which works quite well
- Phenobarbital 60-120mg or 2-4 mg/kg

3.9 DENTAL HEALTH

W.Boardman, E Dubois, J. Felder. Reviewed By S.Unwin

Chronic dental and gingival disease can give rise to potentially life threatening illnesses, thus the importance of dental health should never be underestimated. During routine physical examinations (e.g.: whilst primates are sedated for TB testing during quarantine or subsequently) close attention should be paid to the condition of teeth and gums. Any appropriate treatment should be instituted as soon as possible.

DENTAL FORMULA OF ADULT CHIMPANZEES AND GORILLAS
2-1-2-3
2-1-2-3

DENTAL ERUPTION CHART

Species	Type	Incisors	Canines	Premolars	Molars
Chimpanzee	Deciduous	60-200 days	256-565 days	UNK	84-431 days
	Permanent	59-92 months	96-121 months	73-100 months	36-163 months
Gorilla	Deciduous	UNK	UNK	UNK	UNK
	Permanent	64-90 months	77-124 months	71-103 months	36-157 months

3.10 COMMON PARASITIC DISEASES DIAGNOSIS AND CONTROL

Wendi Bailey and Steve Unwin

This chapter is designed as an overview, highlighting potentially severe parasitic infections, based on disease of concern risk analysis. Further and more in depth information can be found in Cheeseborough (2005, in press) and at several websites (refer to the reference section). Life cycle diagrams in this chapter were sourced from http://www.biosci.ohio-state.edu/~parasite

General Guidelines

- All these diseases are zoonoses and must be managed as such
- Routinely check stools every 3 months. See section 3.17 for the formol-ether concentration technique for gastrointestinal parasites.
- Recheck 7-10 days after treatment to monitor effectiveness.
- Keep microscopy equipment, including slides, away from humidity.

A. GASTROINTESTINAL NEMATODES

1. HOOKWORMS – ANCYLOSTOMA SPP, NECATOR SPP.

- Heavy infection can cause anaemia – especially in juveniles
- Causes bloody diarrhoea
- Primarily a human pathogen
- Direct life cycle – no intermediate host - Refer to Life Cycle diagram (figure 4) for more details
- 1 week is required for infective larvae to develop from eggs in the environment
- Strict Hygiene and vector control are vital to prevent spread
- Diagnosis based on finding eggs in the stool (figure 1 and 2) BUT larvae can hatch quickly in high ambient temperatures and should be differentiated from strongyloides L1 larvae. Hookworm L1 larvae have a long buccal cavity (figure 3).
- Ivermectin, mebendazole, albendazole or possibly levamisole at standard doses can be used for treatment. Treatment for concurrent anaemia may also be required.

Hookworm eggs are standard 'strongyle' eggs – oval, thin shelled, relatively large (65 x 40µm). When identifying eggs, you cannot distinguish different hookworm species from each other, or from other strongyle infections, such as Oesophagostomum spp. Treatment however is the same.

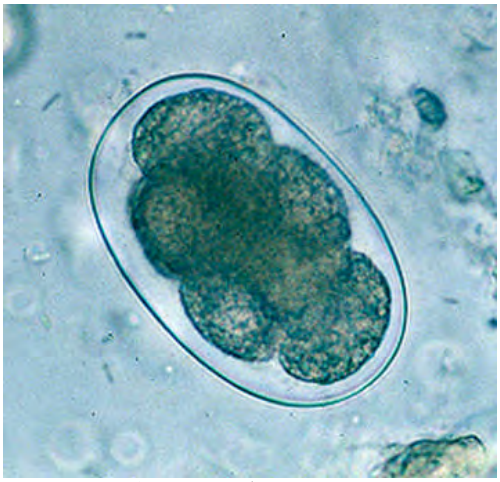


Figure 1. Hookworm spp egg

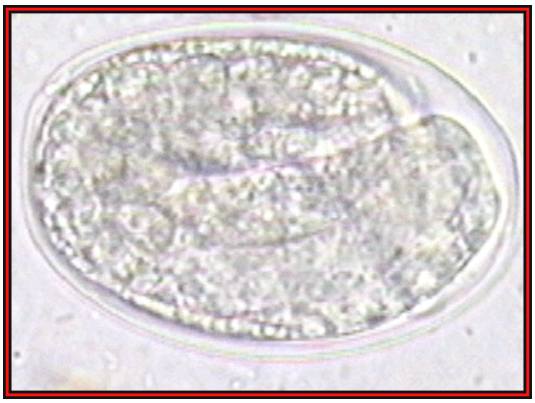


Figure 2. Larvated hookworm egg

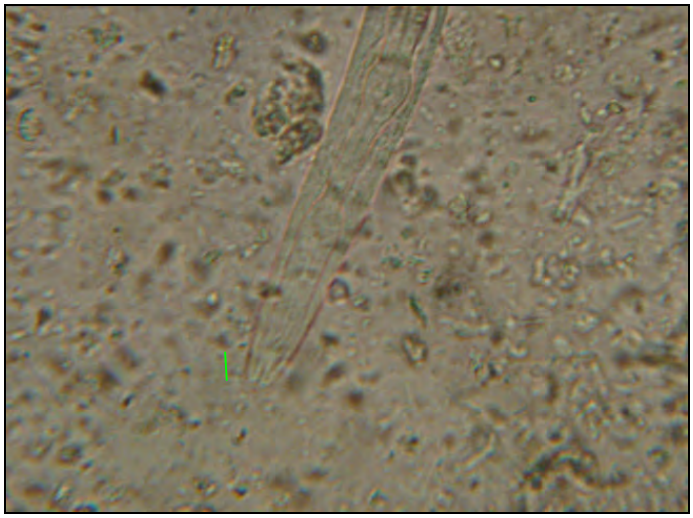


Figure 3. Buccal cavity (green line), L1 larva - hookworm

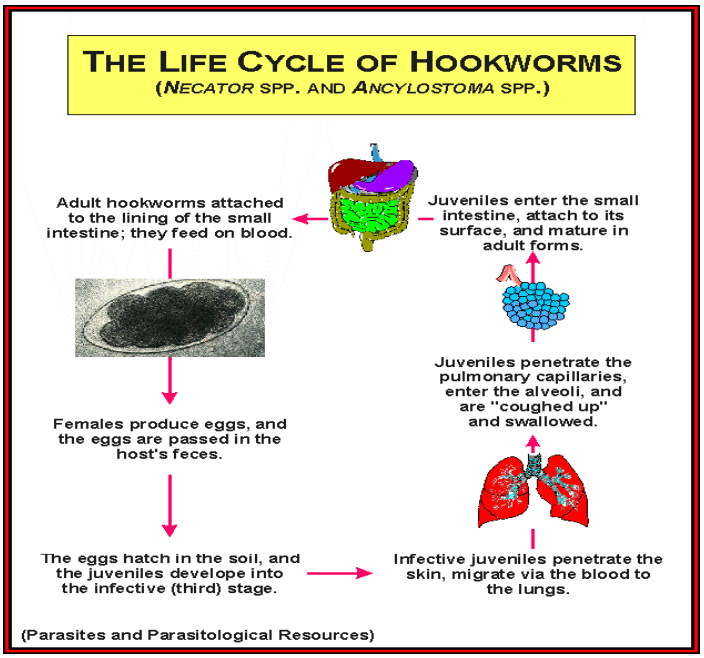


Figure 4. Hookworm life cycle

2. ASCARIDS

- Potentially can induce sudden death due to blockage – individual worms can grow up to 18 inches long (figure 5).
- Infection is frequently asymptomatic
- Eggs adhere to many surfaces and become mixed in soil and dust
- Primarily a human pathogen
- Direct life cycle (figure 6)
- Strict hygiene
- Vermin control
- Piperazine, ivermectin, mebendazole, pyrantel, fenbendazole, albendazole are all effective wormers.
- Egg 50-70 x 30-50um. yellow brown colour, “bumpy” coat (may be decorticated)

Figure 5. A heavy burden of ascarids can cause intestinal blockages

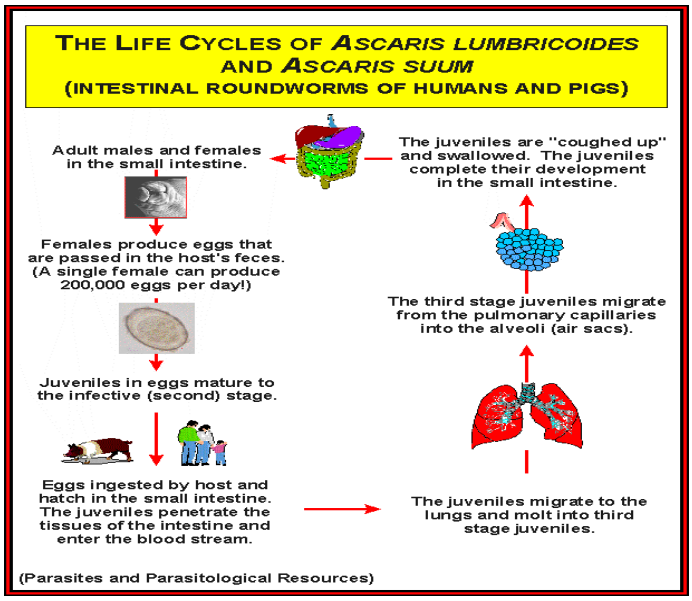


Figure 6. Ascarid life cycle

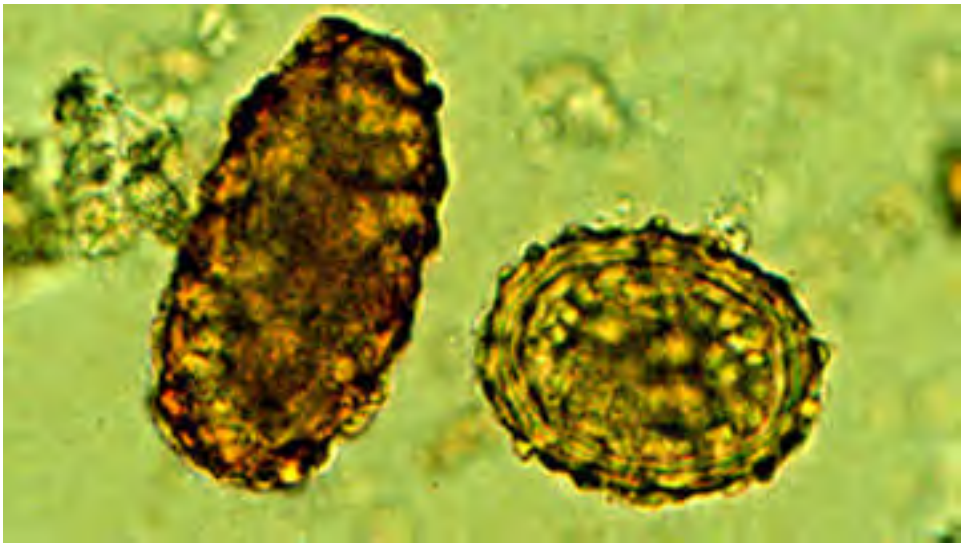


Figure 7. Ascarid eggs. Left: infertile (90um) Right: fertile (60-75um)

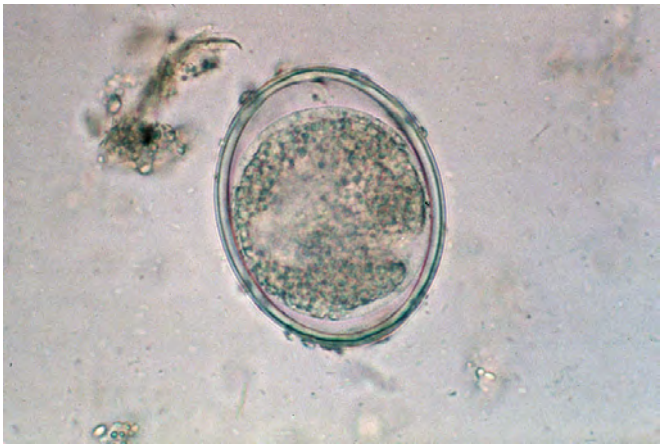


Figure 8. Decorticated, fertile (note double wall)

3. TRICHURIS (WHIPWORM)

- **Heavy infections result in colitis (+/- secondary bacteria and/or protozoa) (figure 9)**
- Direct life cycle (figure 10)
- Strict hygiene. The eggs are highly susceptible to desiccation, so keep areas as dry as possible
- Pest control is important to minimise spread
- Anthelmintics: mebendazole, albendazole, ivermectin, flubendazole, pyrantel/oxantel combo.
- Egg: must differentiate from Capillaria (has non-protruding polar plugs, size 45x21um) (figure 12)

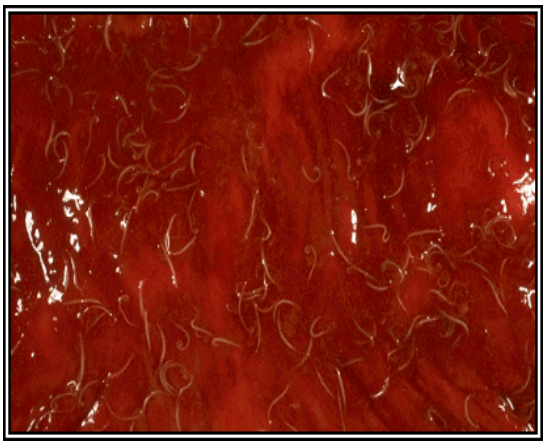


Figure 9. Colitis induced by a heavy Trichuris infection.

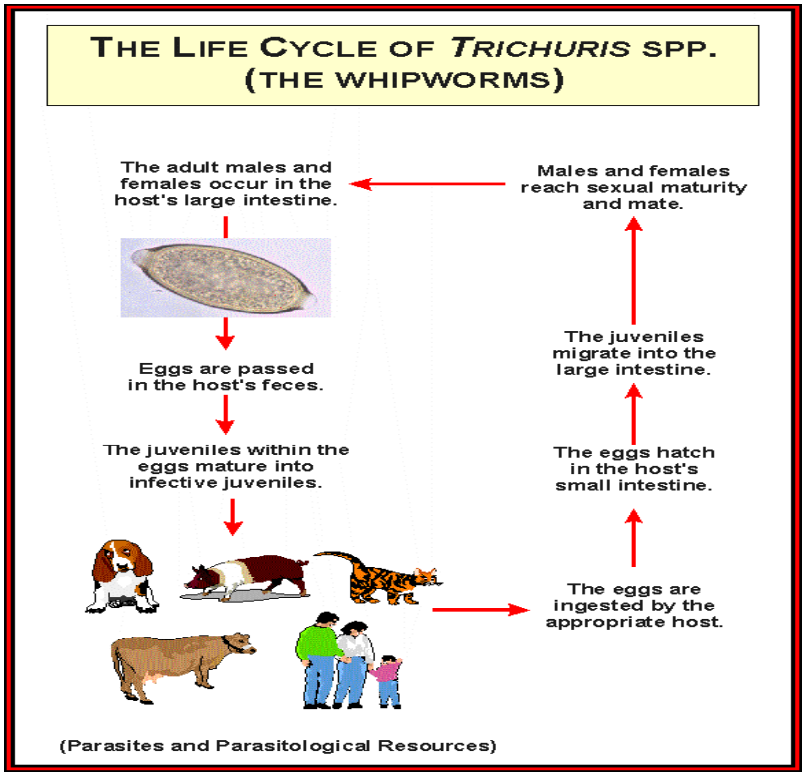


Figure 10. Trichuris life cycle

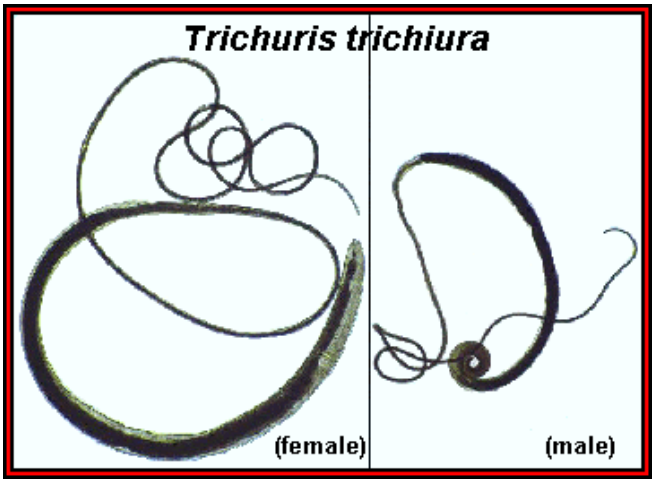


Figure 11. Adult Trichuris (whipworm)

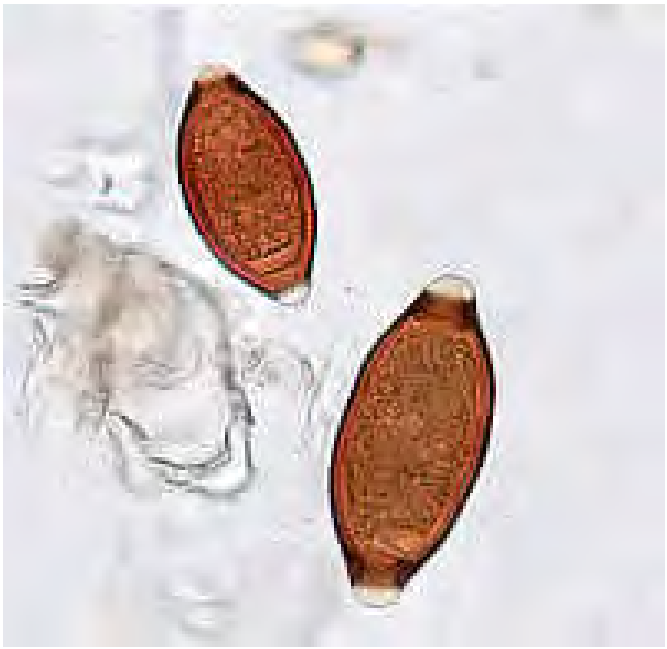


Figure 12 . Trichuris Eggs,50x25µm

4. ENTEROBIUS SPP (PINWORMS)

Figure 13 Pinworm Male

Figure 14. Pinworm Female 8-13mm

- Infection is often asymptomatic but anal pruritis also common
- Fatal cases reported in chimpanzees
- Direct life cycle. (figure 16).
- Ova can spread in air/ dust
- Strict hygiene essential
- Human to animal spread easy and common
- Effective anthelmintics: Mebendazole, Albendazole, pyrantel

Figure 15. Pinworm Egg, oval, larvated flattened on one side. 55x30µm

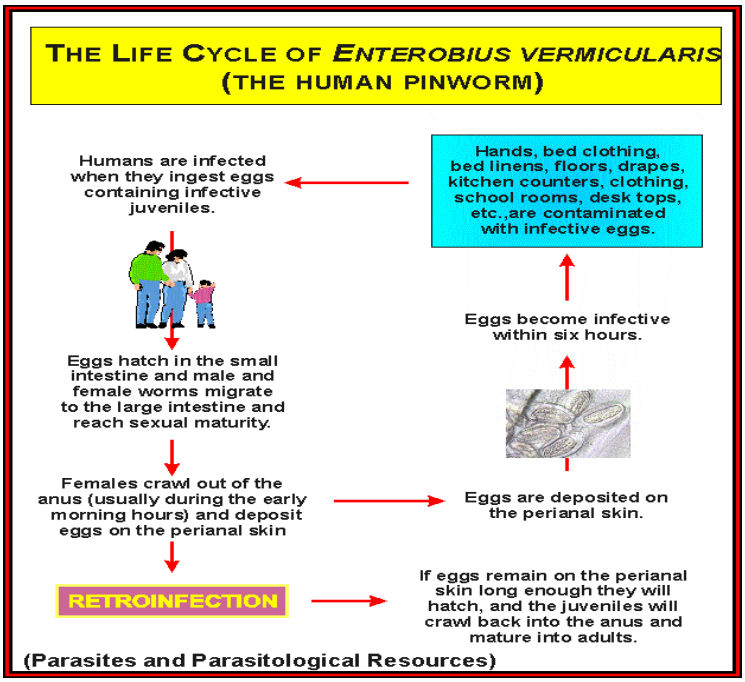


Figure 16. Pinwom life cycle

5. STRONGYLOIDES SPP

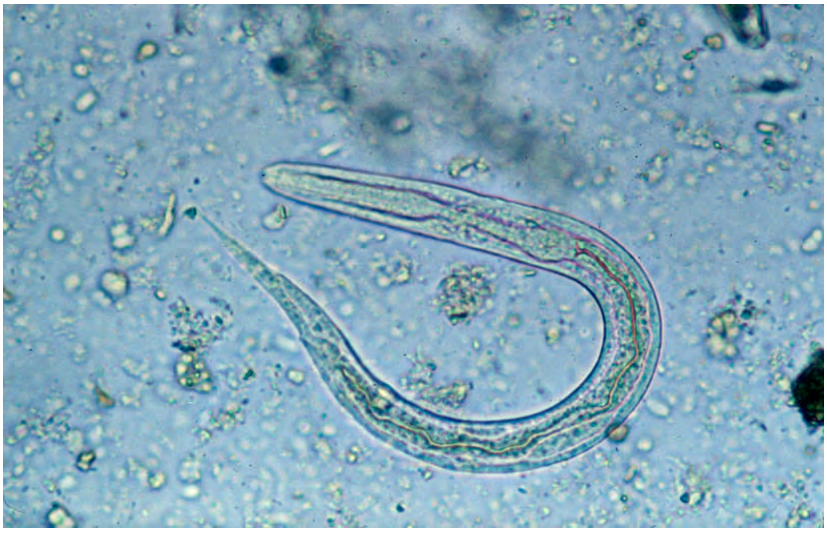


Figure 17. Strongyloides species L1 (rhabditiform) larva- short buccal cavity. Unsheathed Size: 200-280x 16µm

- Clinical signs highly variable. Often asymptomatic – especially in adults. Mucoid or Haemorrhagic diarrhoea. In chronic infections – progressive weight loss and weakness (no diarrhoea)
- Death is usually a result of pneumonia and peritonitis, due to a sudden and massive increase in L1 larval migration, due to concurrent immunosuppression.
- *S.stercoralis* in humans and apes
- *S.fulleborni* in chimps, baboons, guenons
- Various species in monkeys
- Complex life cycle - Parasitic and free living (figure 24)

- Strict hygiene – maintain enclosures as dry as possible to prevent free living stages.
- Anthelmintic possibilities: Ivermectin, thiabendazole, mebendazole, levamisole, pyrantel. A single dose of ivermectin or albendazole, or multiple doses of thiabendazole may give good results against the intestinal stages, but are ineffective against parental stages. Multiple treatments with ivermectin at a dose rate of 0.4mg/kg may be necessary to control this worm.
- Diagnosis: L1 larva with short buccal cavity, figure 17, (larvated egg if *S.fuelleborni*), culture for characteristic L3 larvae (unsheathed, size around 600µm)

Figure 18. S.fuelleborni egg 50x35µm

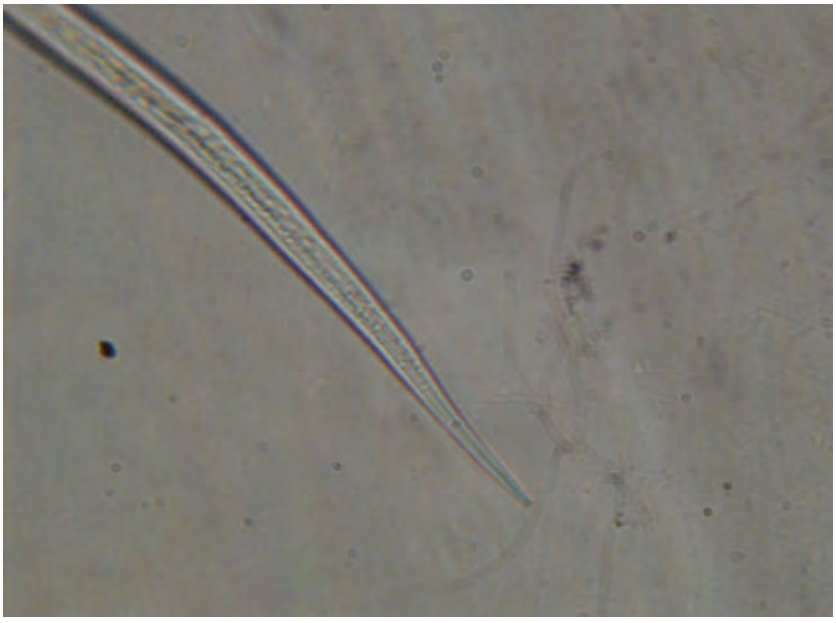


Figure 19. Strongyloides spp L3 larva, ***notched posterior end***

Figure 20 and 21. Free-living strongyloides spp adult male, note curved posterior end

Figure 22 and 23. Adult female Strongyloides fuelleborni, note "waist" and vulval lips

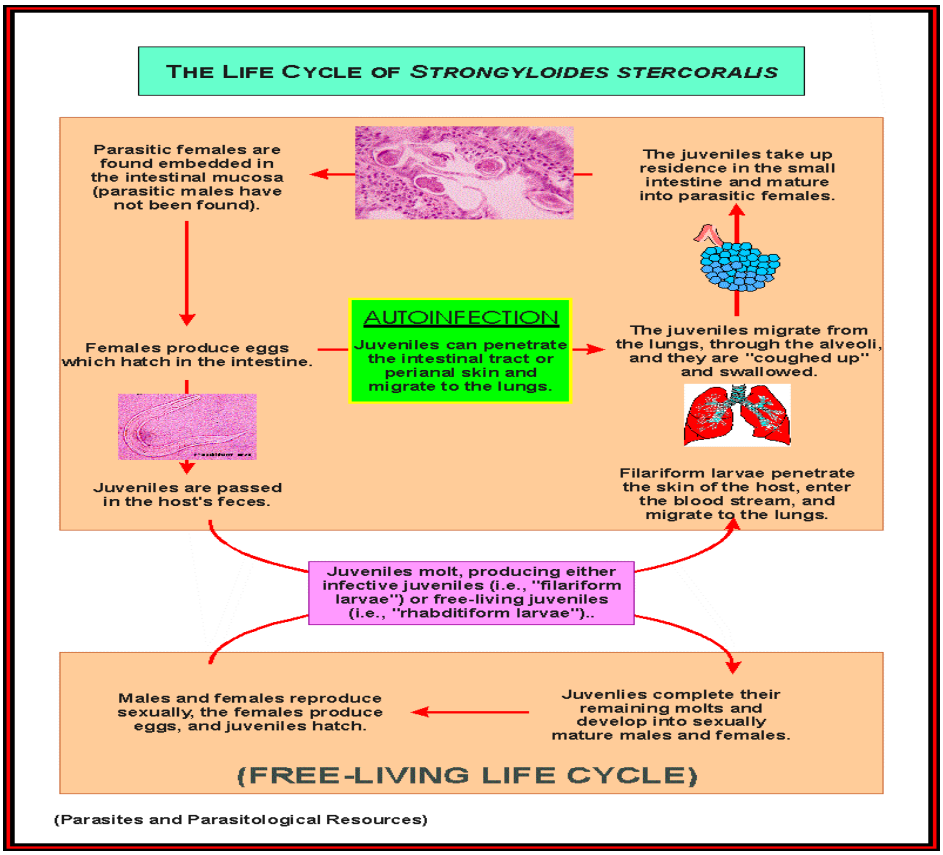


Figure 24. Stongyloides spp. Life cycle

6. OESOPHAGOSTOMUM Spp.

Oesophagostomum bifurcum is the most-common species infecting humans in Africa – especially in Togo and Ghana. This nematode has been the confirmed cause of death in drills and gorillas, and has been found in chimpanzees, in PASA sanctuaries. The majority of this section comes from the CDC website, as most of the photos from PASA show pathological changes, rather than the worms themselves. This worm is primarily a parasite of **monkeys.** Figure 25 shows the life cycle.

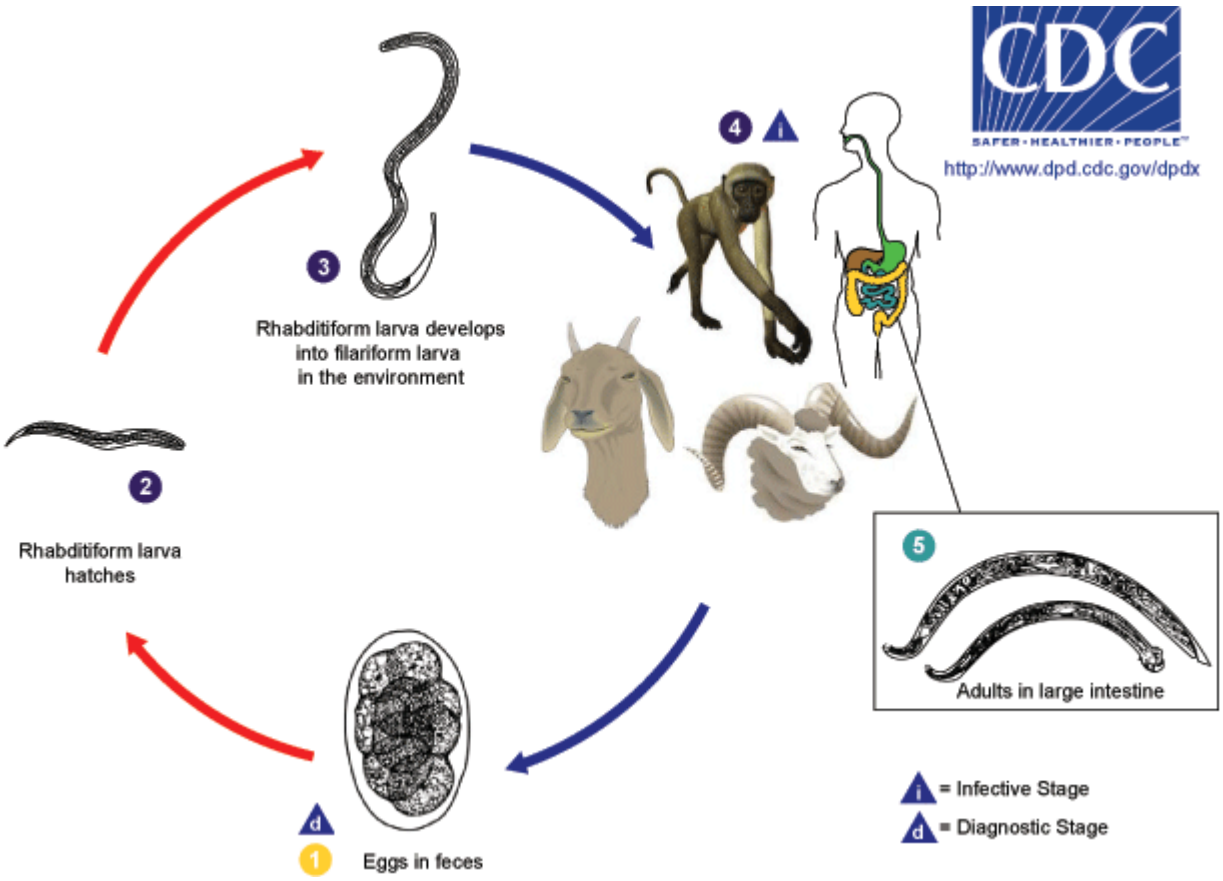


Figure 25. Oesophagostomum life cycle. Common livestock such as sheep, goats, and swine, as well as non-human primates, are the usual definitive hosts for *Oesophagostomum* spp., but other animals, including humans and cattle, may also serve as definitive hosts. Eggs are shed in the faeces of the definitive host 1, and may be indistinguishable from the eggs of *Necator* and *Ancylostoma*. Eggs hatch into rhabditiform (L1) larvae in the environment 2, given appropriate temperature and level of humidity. In the environment, the larvae will undergo two moults and become infective filariform (L3) larvae 3. Worms can go from eggs to L3 larvae in a matter of a few days, given appropriate environmental conditions. Definitive hosts become infected after ingesting infective L3 larvae 4. After ingestion, L3 larvae burrow into the submucosa of the large or small intestine and induce cysts. Within these cysts, the larvae moult and become L4 larvae. These L4 larvae migrate back to the lumen of the large intestine, where they moult into adults 5. Eggs appear in the faeces of the definitive host about a month after ingestion of infective L3 larvae.

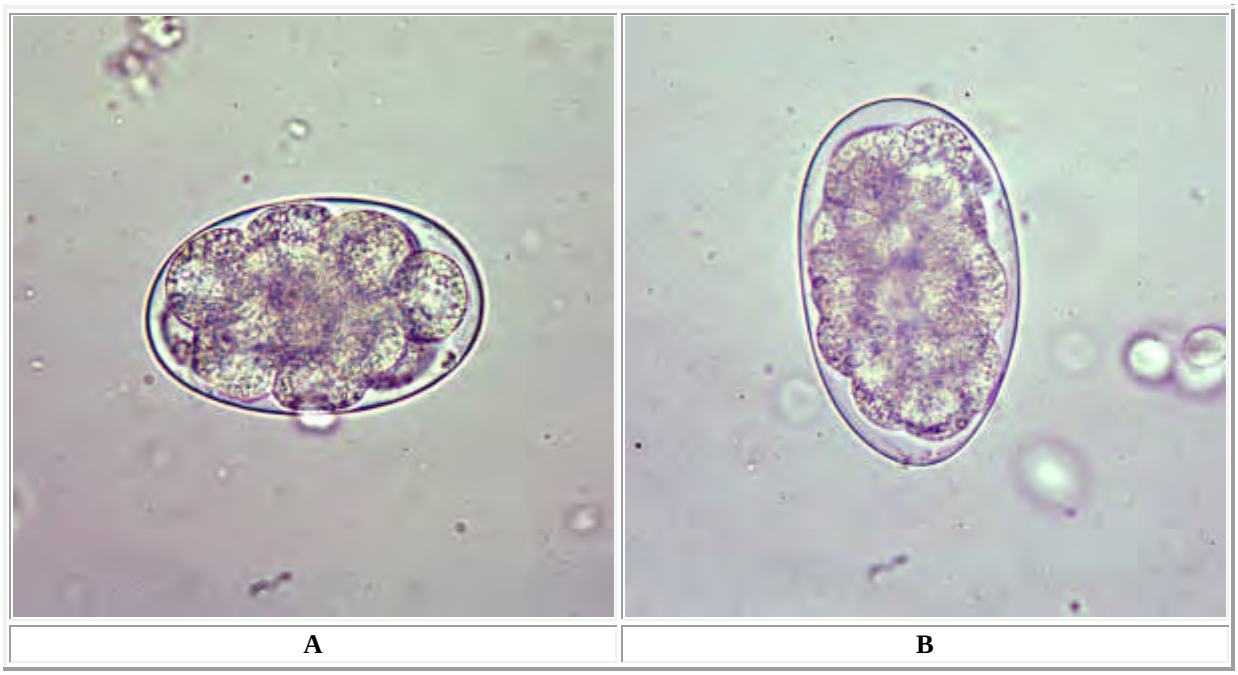
Clinical signs include:

- Acute abdomen is the most-common manifestation in humans, mimicking an appendicitis.
- A low-grade fever and tenderness in the lower-right quadrant are the most-common symptoms; vomiting, anorexia, and diarrhoea are less-common.
- Intestinal obstruction may also occur, mimicking a hernia.

- In rare instances, Oesophagostomum spp. will perforate the bowel wall, causing purulent peritonitis or migrate to the skin, producing cutaneous nodules. These nodules have been found in chimpanzees

As a typical strongyle, like hookworm, diagnosis can be difficult based on worm egg examination alone, as they look the same. Eggs tend to be shed in greater numbers during cases of oesophagostomiasis than hookworm infection, however. Finding an intact worm during surgery or in a biopsy specimen can provide a definitive diagnosis.

The eggs of *O. bifurcum* measure 60-75 µm long by 35-40 µm wide (Figure 26). Eggs are often in a later stage of cleavage (increased cellular bifurcation within the egg), than hookworm species when shed in faeces.



A	B

Figure 26 A and B. *Eggs of Oesophagostomum sp. in an unstained wet mount of stool.*

Adults of *Oesophagostomum* spp. are bursate nematodes, related to and morphologically-similar to, the hookworms. Females measure 1.5-3.0 cm in length; males are smaller. In both sexes, the anterior end has a cephalic inflation or vesicle, a transverse cephalic groove, and an oral opening guarded by external and internal leaf crowns (corona radiata) (Fig 27 - 29). The posterior end of the female is short and pointed; the male possesses a symmetrical bursa and paired, equal spicules. Adults reside in the **large intestine** of the definitive host (Hookworm in the small intestine).

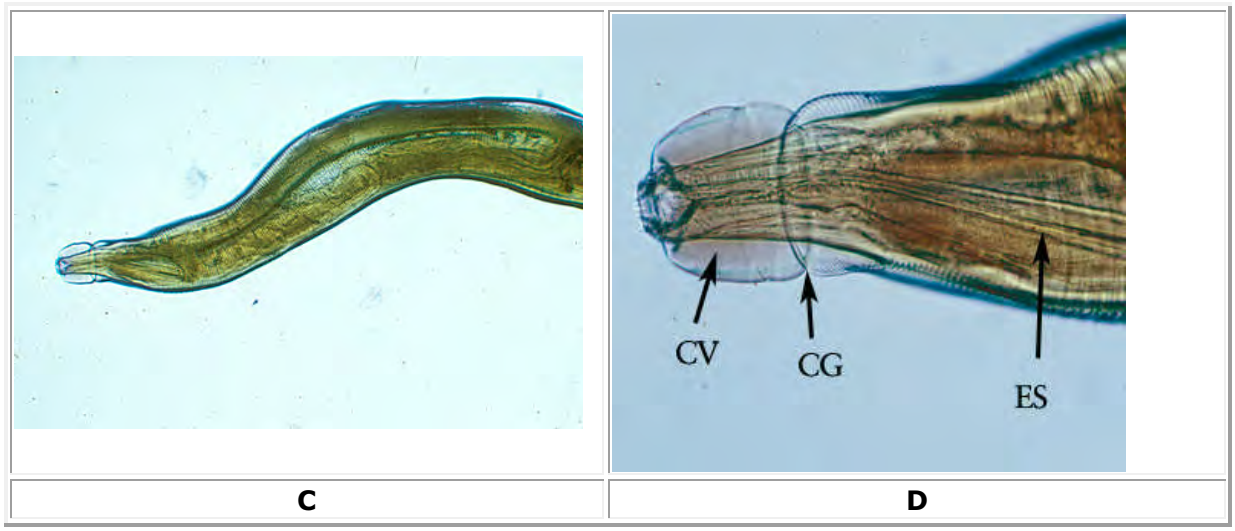


Figure 27 C and D. C: *Adult of Oesophagostomum sp.* ***D:*** *Higher magnification of the anterior end of the specimen in Figure* ***C****. Note the presence of the cephalic vesicle (****CV****), cephalic groove (****CG****) and esophagus (****ES****).*

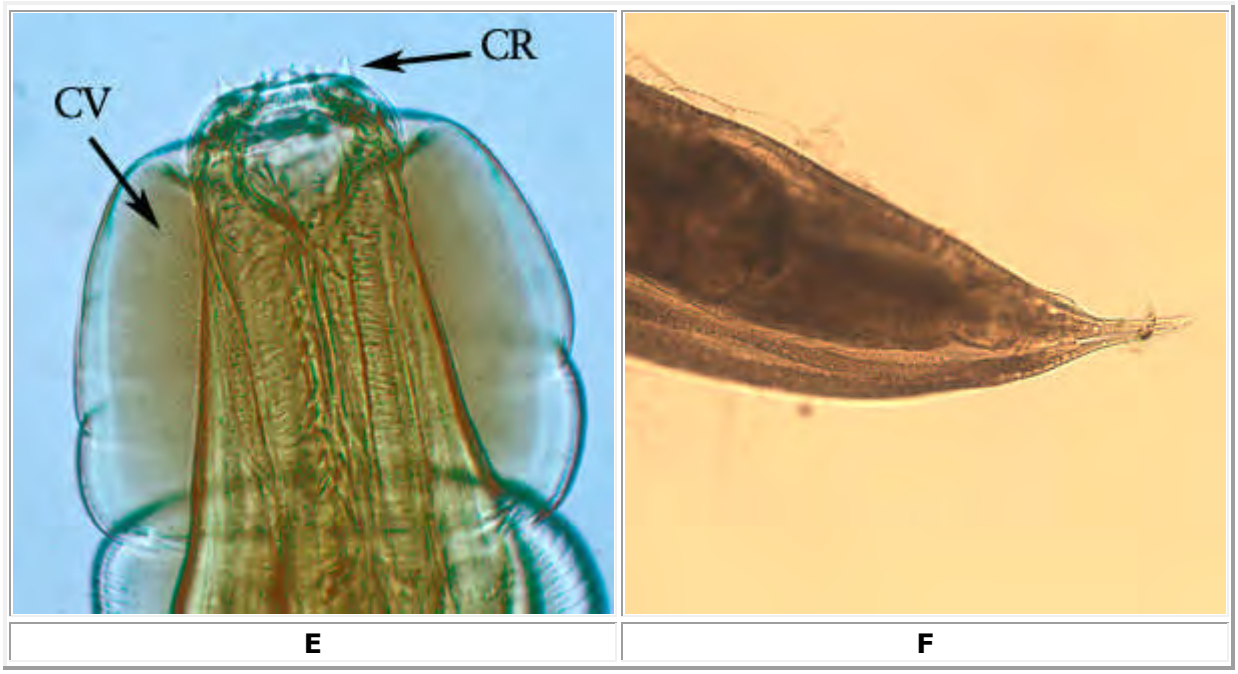


Figure 28 E and F. E: *Higher magnification of the anterior end of the specimen in Figures 27. Note the presence of the cephalic vesicle (****CV****) and corona radiata (****CR****).* ***F:*** *Posterior end of a female Oesophagostomum sp., showing the pointed tail.*

Figure 29 G and H. *Posterior end of a male Oesophagostomum sp., shown in two different focal planes. Note the spicule (**SP**) and bursa (**BU**).*

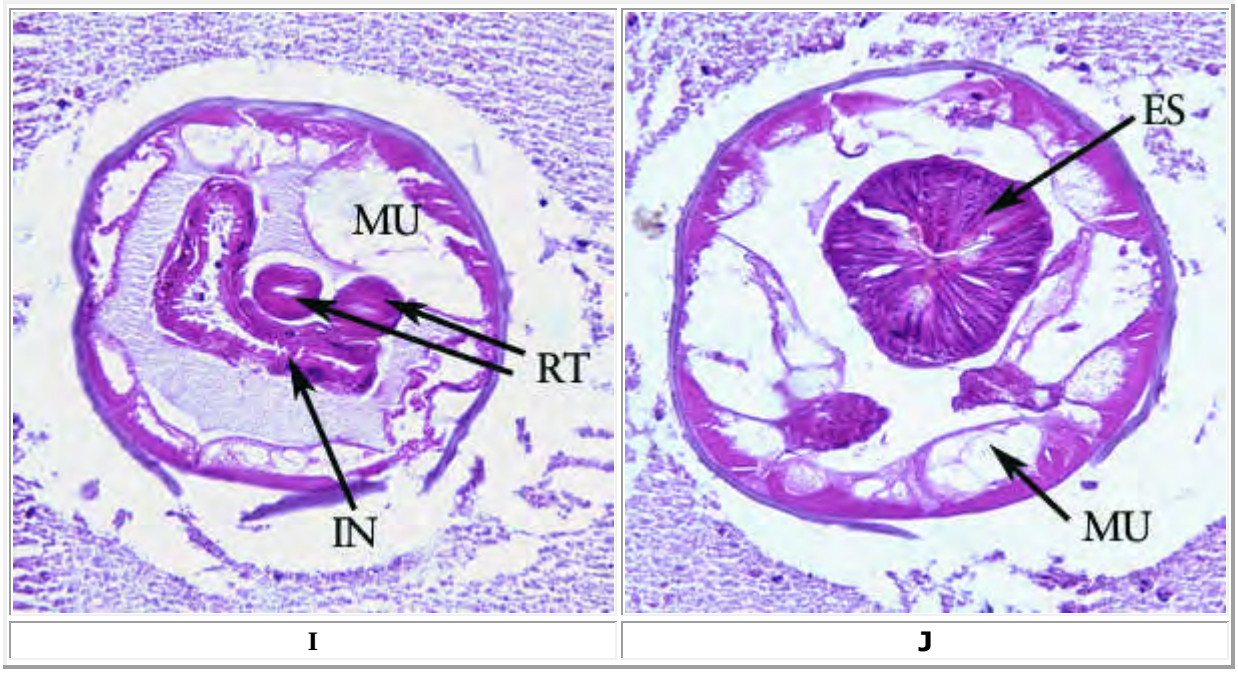


Figure 30 I and J *Cross-sections of an adult of Oesophagostomum sp. in a colon biopsy specimen from a patient from Africa, stained with hematoxylin and eosin (H&E). Note the large, platymyarian muscle cells (**MU**), intestine with brush border (**IN**), paired reproductive tubes (**RT**), and thick, muscled esophagus (**ES**). Images taken at 200x magnification.*

Treatment is usually limited to the surgical removal of adult worms from tissue. Albendazole has been shown to be the most effective antihelminthic drug for the removal of worms from the lumen of the large intestine.

B. **FILARIASIS**

- Intermediate host – blood sucking insects
- In chimps, microfilariae remain in the dermis – *Mansonella streptocerca/ rodhaini* (in other species, because of the life cycle periodicity check blood for microfilaria at night).
- Diagnosis thick blood film (20µl drop) or membrane filtration (3.0µm pore)
- Usually asymptomatic – occasional skin disease
- Potential treatment - Diethylcarbamazine - dangerous

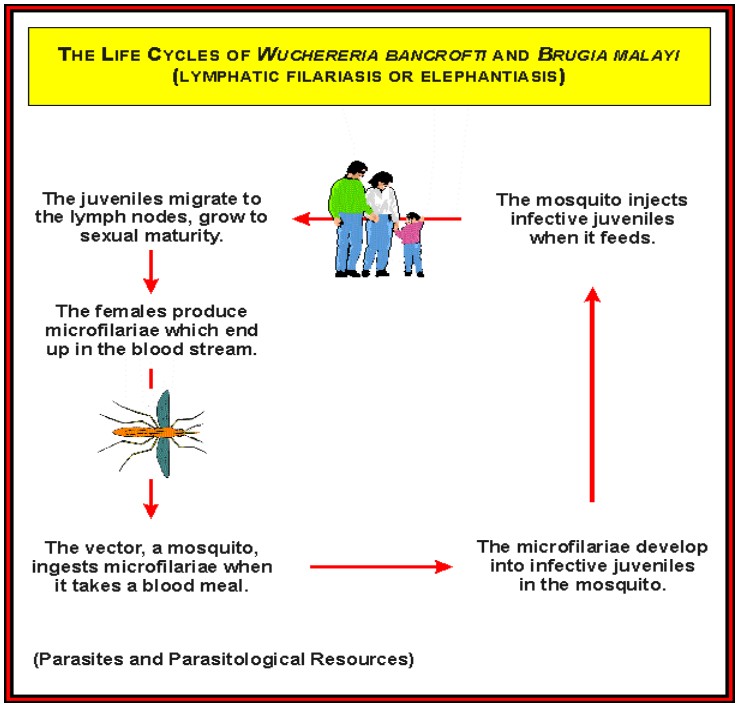


Figure 31. Filariasis life cycle

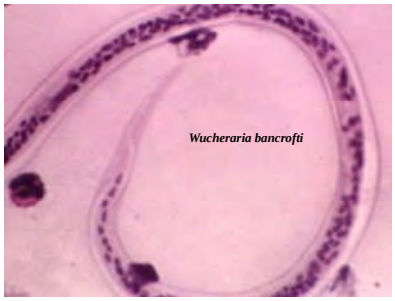


Figure 32. W.bancrofti
275-300 x 8-10um
Sheathed, nuclei do NOT extend to tail tip
Nocturnal periodicity
In blood

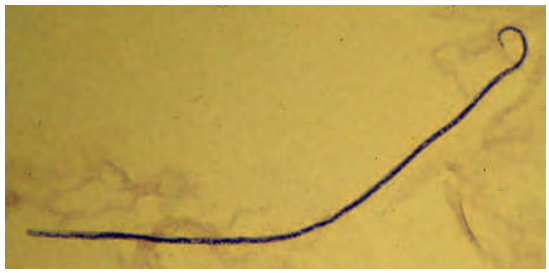


Figure337. M.streptocerca
180-240 x 5um
Unsheathed, nuclei extend to tail tip which is often hooked

In skin

C. SCHISTOSOMIASIS

- **Intestinal disease** caused by *S.mansoni, S.intercalatum, S.japonimcum*- paired, mature adult flukes in venule of rectum and lower L.I.
- **Urinary disease** caused by *S.haematobium* – paired, mature adult flukes in veins surrounding bladder and may be found in veins of liver and rectum
- **Intermediate host :snails**
- **Transmission:** contact with water infected with cercariae. Potentially a big problem for staff in endemic areas.
- **Diagnosis-**faecal concentration technique usually required for intestinal schistosomes, membrane filtration (12.0µm pore) of urine.
- **Treatment –** Praziquantel is effective against all types. Oxamniquine is cheaper, but only effective against *S.mansoni*. Metrifonate can be used for *S.haematobium*. Total cure is unfeasible in endemic areas due to the high rate of reinfection.

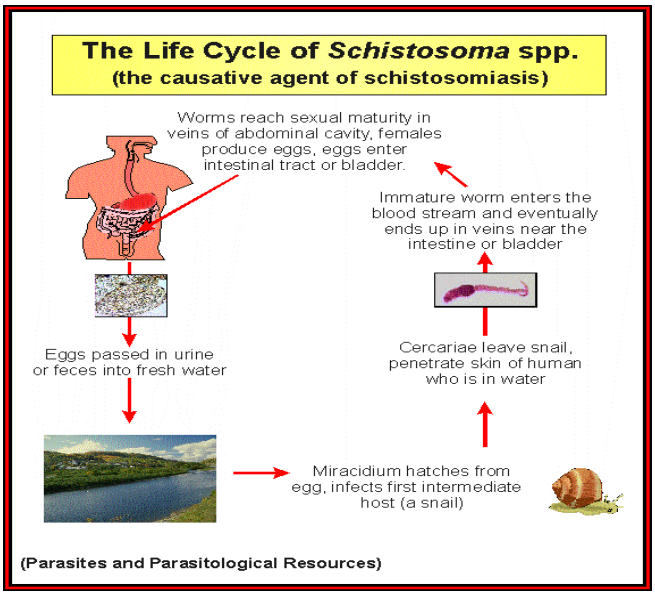


Figure 34. Schistosoma life cycle

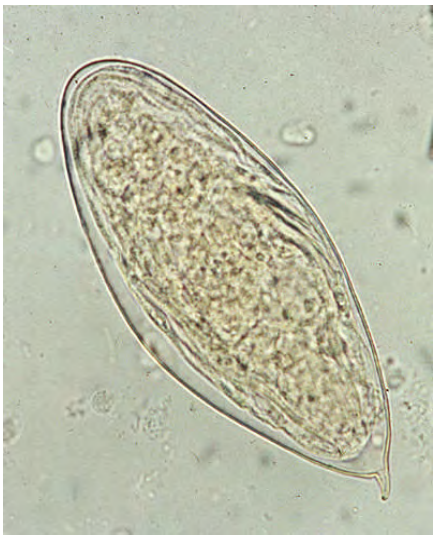


Figure 35. S.haematobium, terminal spine, 145 x 55µm

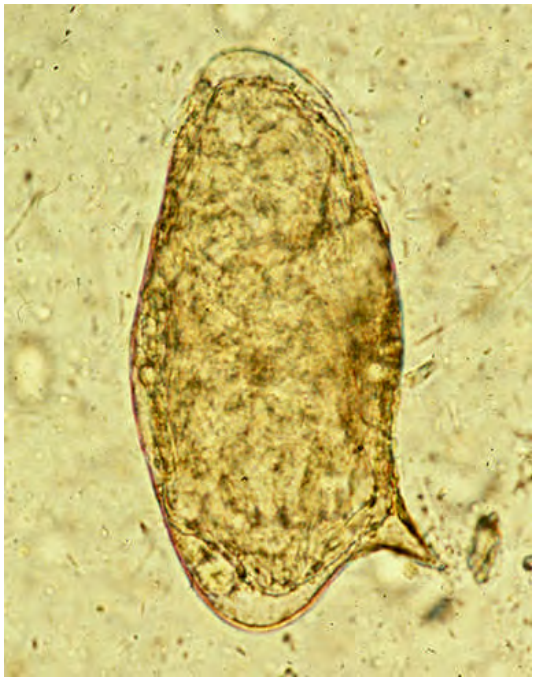


Figure 36. S.mansoni, lateral spine, 150 x 60µm

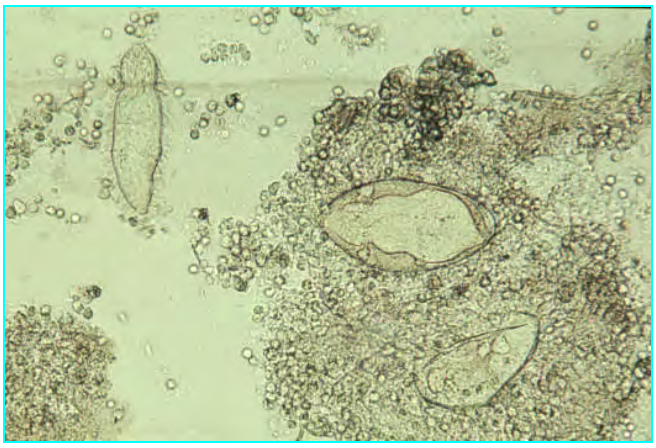


Figure 37. Eggs containing miracidia (and 1 hatched (in seminal fluid)

D. CESTODES

- Taenia spp. eggs are 47-77µm in diameter. They are relatively heavy, making them difficult to identify with normal flotation techniques
- Strict hygiene with effective rodent and insect prevention and control
- Adult tapeworms are not a big problem but due to their size, there is the possibility of intestinal blockage.
- Diagnosis of species by examination of gravid proglottid: size and number of uterine branches.
- Treatment: Praziquantel is the drug of choice.

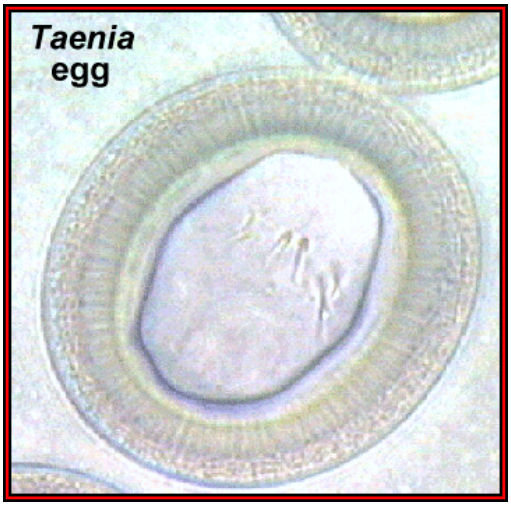


Figure 38. Taenia spp. egg.

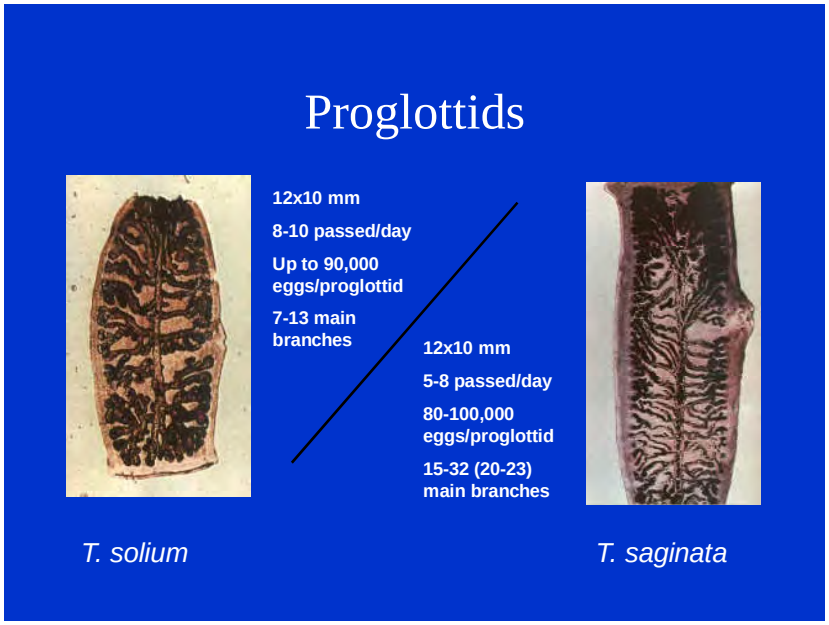


Figure 39. Unstained T.saginata proglottid

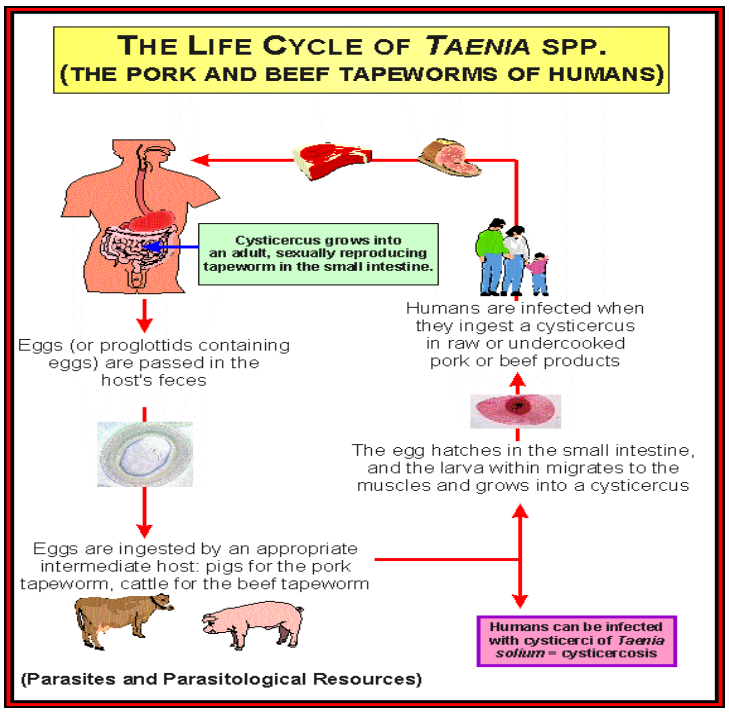


Figure 40. Taenia spp. life cycle.

E. GASTROINTESTINAL PROTOZOA

1. Entamoeba histolytica/ dispar (Amoebic dysentery)

- Opportunistic pathogen – often in the gut in low numbers.
- Only becomes pathogenic if it invades the gut mucosa, so infection is often asymptomatic.
- Clinically affected animals may have mild intermittent diarrhoea through to severe diarrhoea that may be dysenteric or catarrhal. They may be lethargic, show general weakness, dehydration, gradual weight loss, anorexia and vomiting.
- The infective cyst is resistant to drying and many disinfectants.
- Transmission is via food, water, insects and fomites, by ingestion (life cycle fig 49).
- In stool samples cysts of *E. histolytica* and *E. dispar* are identical (up to 4 nuclei around 12.0µm) those of *E.coli* are very similar but have more than 4 nuclei. Only *E. histolytica* can be dangerous – remember all are normal gut flora.
- As is usually a secondary invader, the immune status of the animal is important. So, if identified or implicated in diarrhoea, will very often be another cause for the illness. HOWEVER, if isolated, with clinical signs – will require treatment.
- Samples (mucoid/bloody,fluid) need to be less than 30 minutes old to identify possible *E.histolytica* trophozoites, as drying and low temperatures will kill motile trophozoites.Only *E.histolytica* trophozoites contain ingested RBC's which is the diagnostic feature (figure 41, 46, 47).
- This is a human pathogen so check in contact staff
- Strict hygiene and vector control must be observed.
- Abscessation is possible, especially in the liver, if it invades beyond the gut.
- Signs to death – 7-130 days
- Metronidazole, Tinidazole, paramomycin and secnidazole are the drugs of choice.

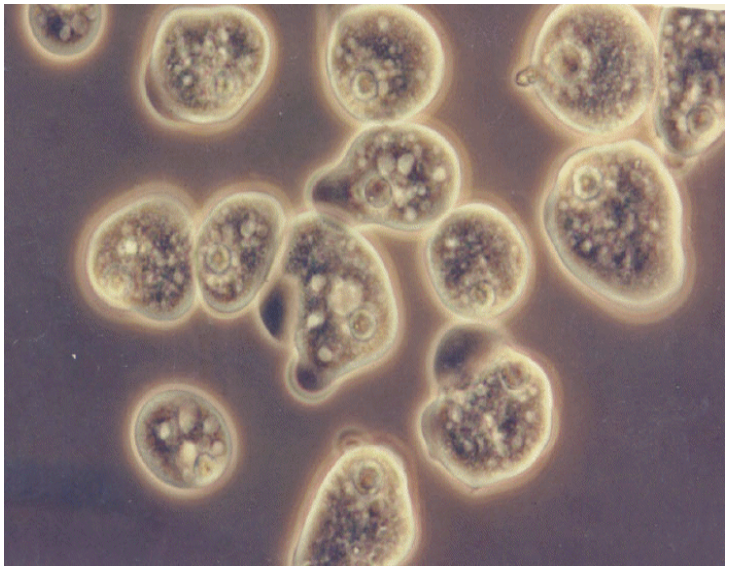


Figure 41. E.histolytica trophozoites (note ingested RBC's)

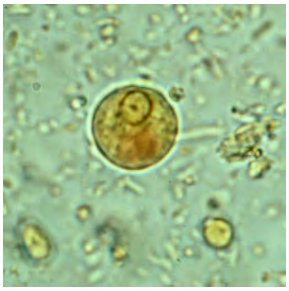
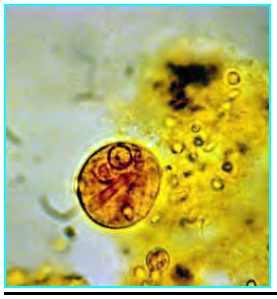
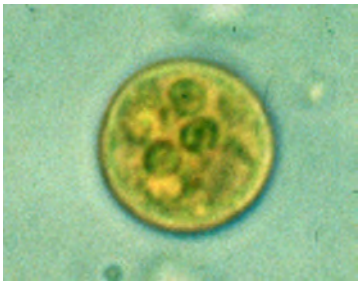




Figure 42 – 44. Entamoeba histolytica/dispar cysts, size range 10-15µm,contain up to 4 nuclei

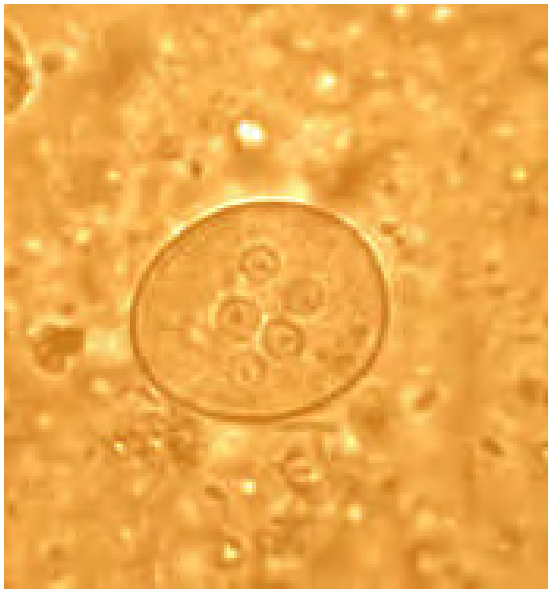


Figure 45. Non pathogenic Entamoeba coli cyst, 10-30 µm , mature cyst has > 4 nuclei

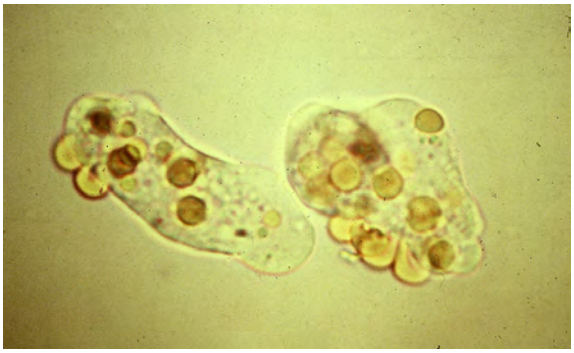
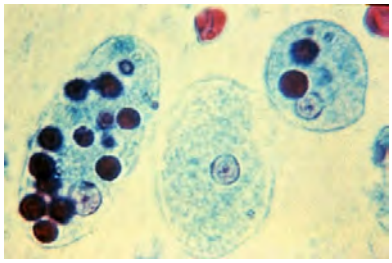



Figure 46 – 47. E.histolytica trophozites, 8-30um, note ingested RBC's. Amoebae show active, directional, amoeboid movement; single nucleus with central karyosome.

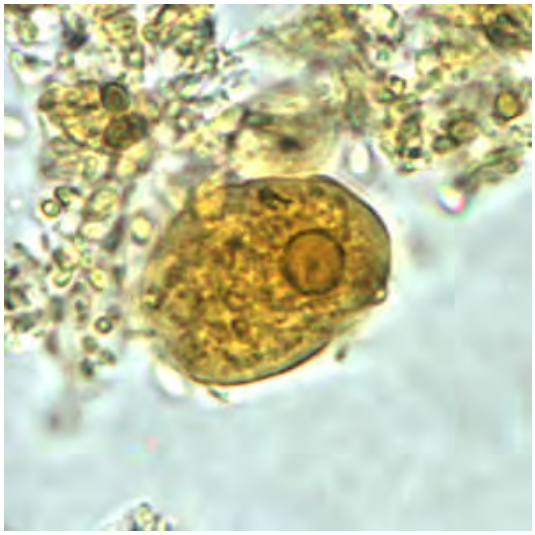


Figure 48. Non pathogenic Entamoeba coli trophozoite, 15-50µm, sluggish, rarely directional movement, nucleus has eccentric karyosome.

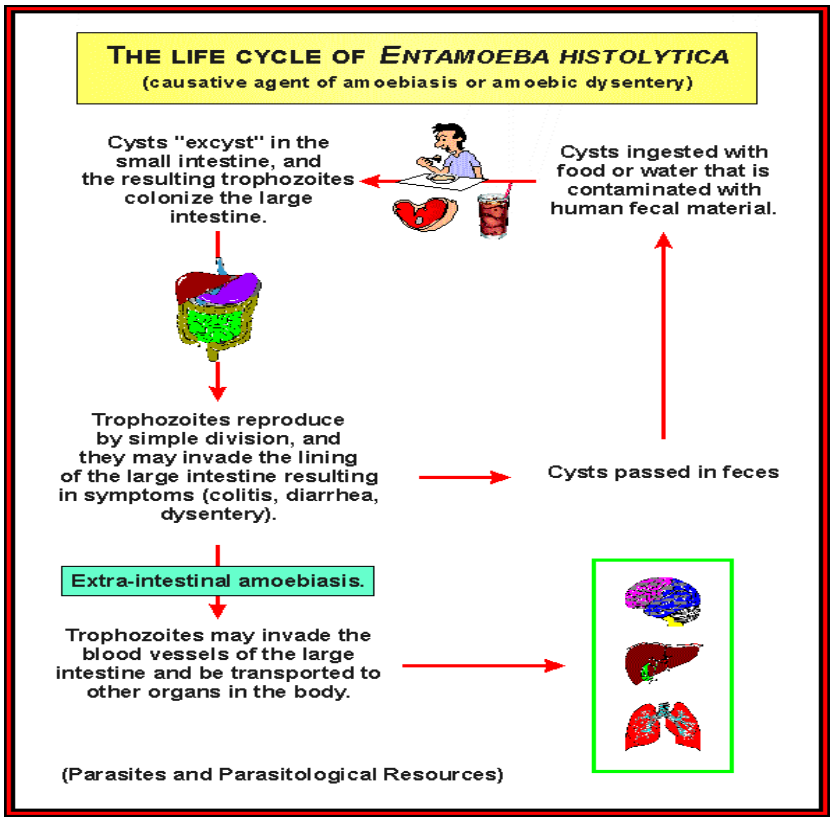


Figure 49. Entamoeba histolytica life cycle.

2. BALANTIDIUM COLI

- Ciliate found in the caecum of primates and pigs.

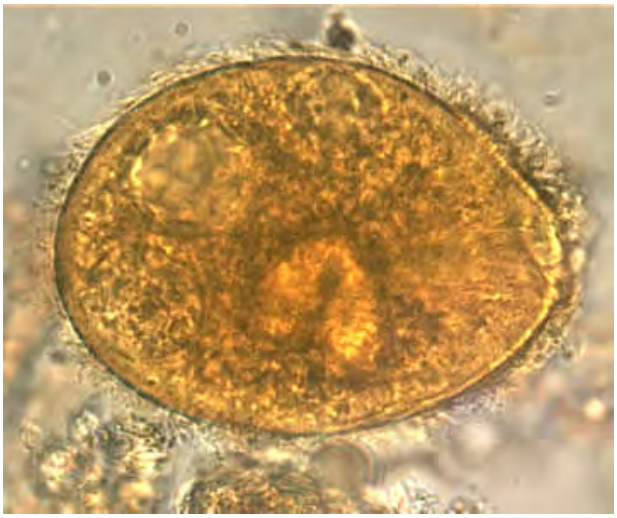


Figure 50. B.coli trophozoite, 50-200 x 40-70µm, large macronucleus and contractile vacuoles may be seen

- Clinically signs include weight loss, anorexia, muscle weakness, lethargy, watery diarrhoea, dehydration, tenesmus, rectal prolapsed. Clinical signs are often self limiting
- Primary pathogenicity not confirmed – (opportunistic pathogens) however, infection in gorillas can progress rapidly to death
- Strict hygiene and vector control must be observed.
- Avoid sudden diet changes, whcihc can change the gut flora, potentially leading to an overgrowth of B.coli.
- Treatment: Metronidazole, paramomycin, tinidazole, doxycycline.

Figure 51. B.coli cyst, trichrome, 50 x 70 µm source dpd.cdc.gov

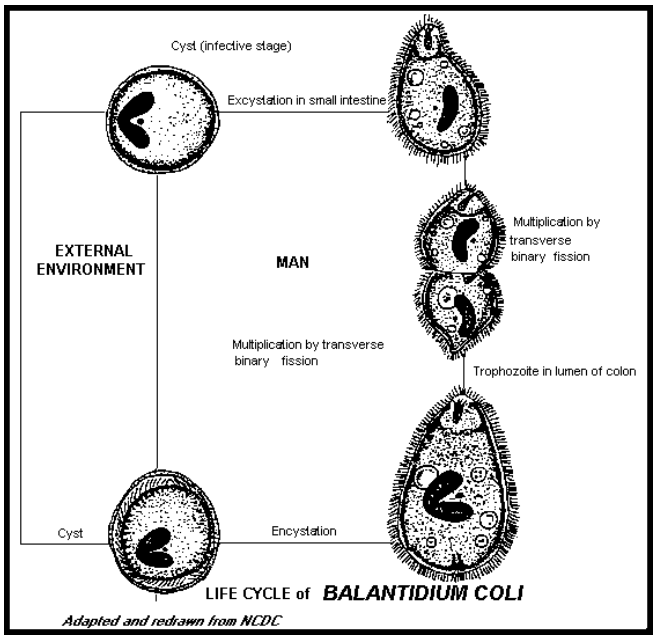


Figure 52. B.coli life cycle

3. GIARDIA

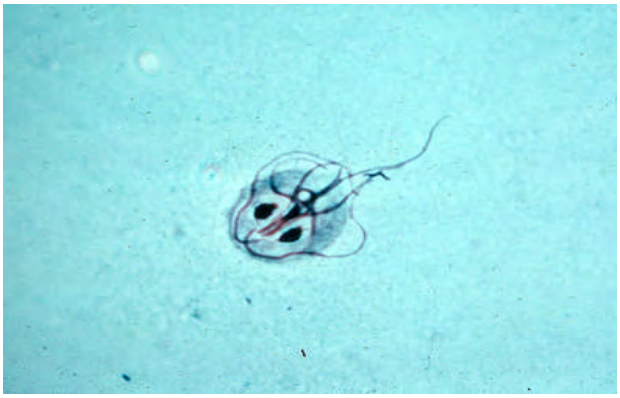
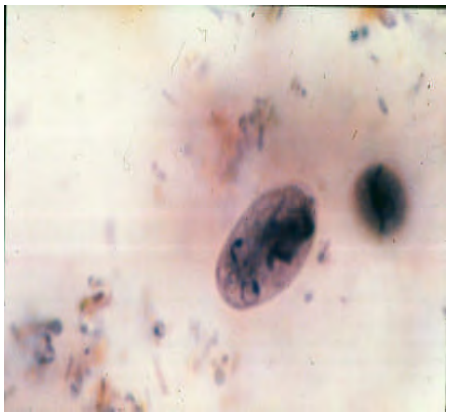


Figure 53. Giardia trophozoite.

- Usually chronic intermittent diarrhoea and weight loss.
- Faeces are poorly formed, rather than watery, and rarely dysenteric. Vomiting is rarely involved. Bouts of diarrhoea (with abdominal pain) of several days duration are common, often with several weeks between bouts.
- Confirm infection with cysts/ trophozoites in faeces
- Cysts oval, 8-15 x 6µm, 4 nuclei, axoneme and remains of flagellae may be seen.
- Trophozoites 12-15 x 5-9µm, rapid tumbling movement (falling leaf), sucking disc on ventral surface, 4 pair flagellae, 2 axonemes & 2 nuceli
- Treatment: Although disease is often self limiting, treatment is recommended, especially in juveniles. Metronidazole resistance is increasing. Tinidazole is preferred, but compliance will be problematic due to the bad taste of both of these medications.



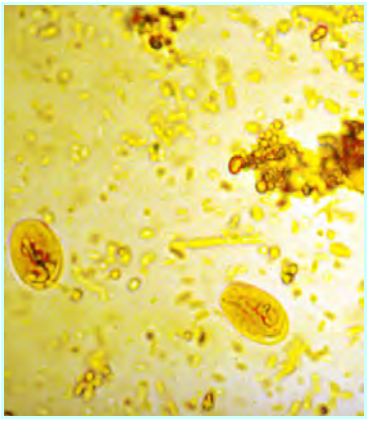


Figure 54 and 55. Giardia cysts, stained with trichrome (Left) and iodine (Right)

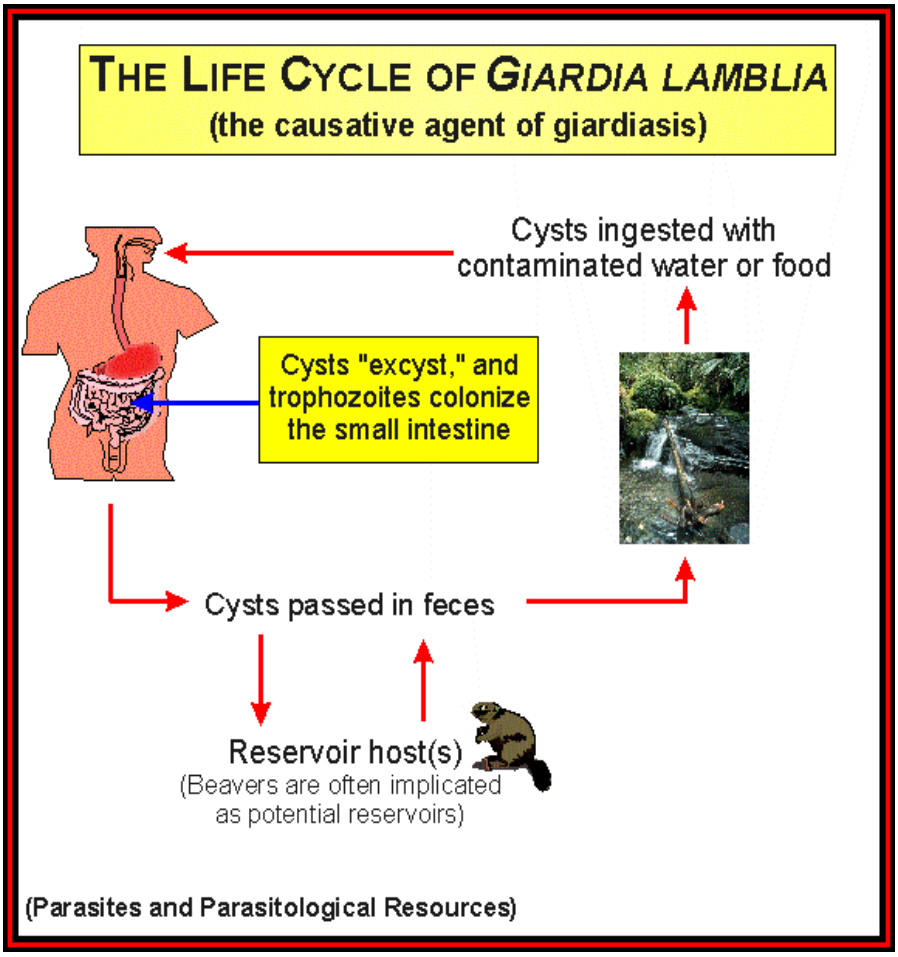


Figure 56. Giardia life cycle

4. BLASTOCYSTIS HOMINIS

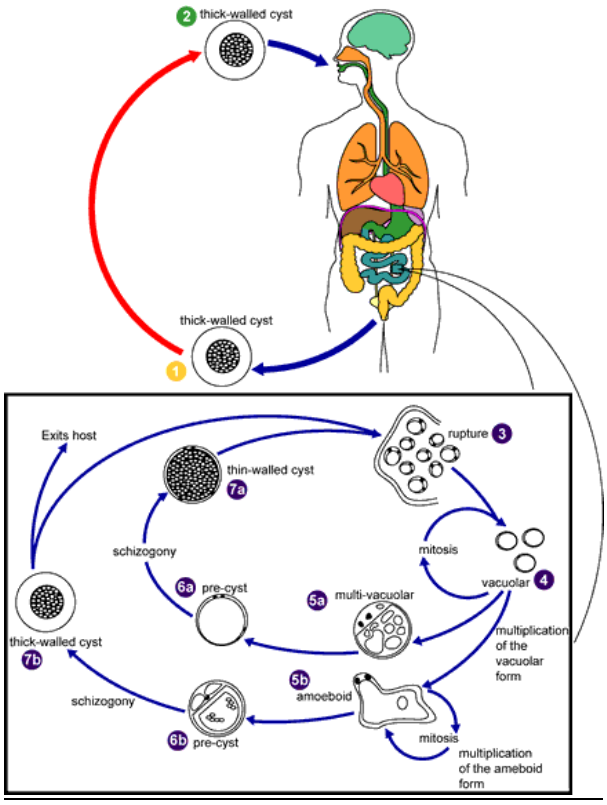


Figure 57. Proposed life cycle for Blastocystis hominis - ? pathogenic

- Infect epithelial cells of digestive tract,multiply asexually.
- Size range 6-40 um (frequently 10um)
- Strong association with *D.fragilis*

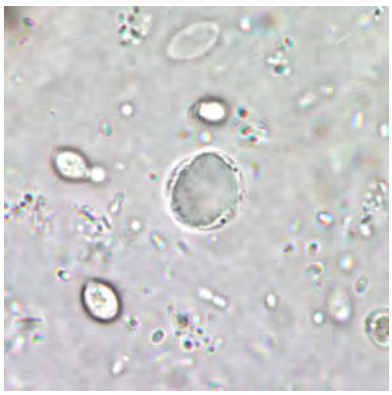


Figure 58 Saline

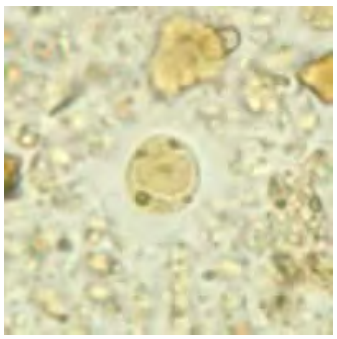


Figure 59 Iodine

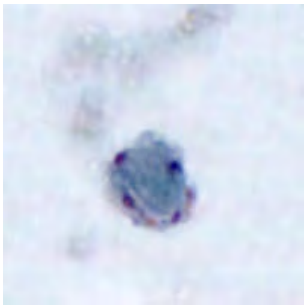


Figure 60 Field's stain

5. DIENTAMOEBA FRAGILIS

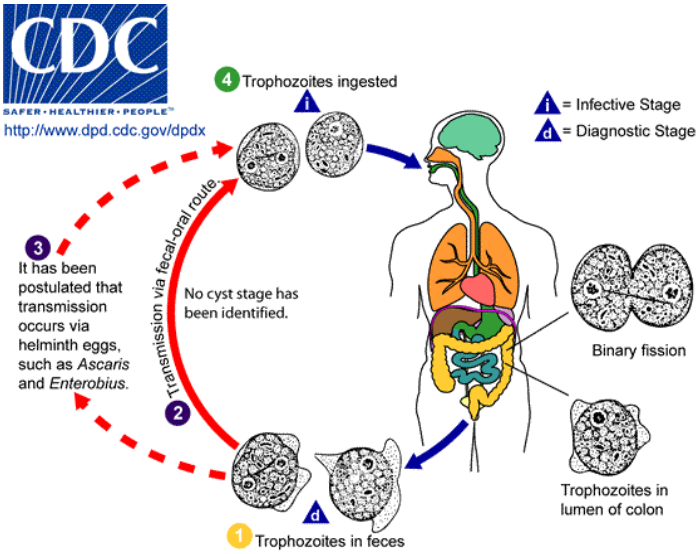


Figure 61. Proposed life cycle for Dientamoeba fragilis

- A flagellate, trophozoite only known stage, 5-15µm
- Need to examine stained preparations
- Uni or bi-nucleate- characteristic diffuse nucleus
- Associated with Irritable Bowel Syndrome?
- Associated with severe gastrointestinal disease in gorillas.

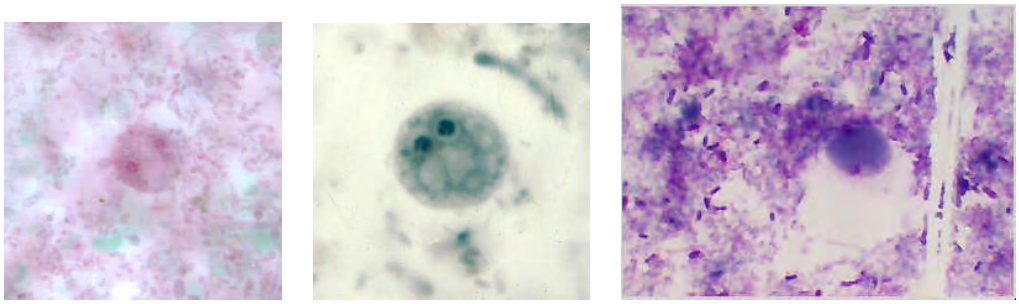


Figure 62 – 64. Various stained preparations of D.Fragilis. Figure 58 is from a gorilla (Limbe). Fields stain.

3.11 MALARIA OF NON HUMAN PRIMATES IN AFRICA: AN AID TO DIAGNOSIS AND TREATMENT.

W. Bailey and S. Unwin

INTRODUCTION AND LIFE CYCLE

Malaria is caused by parasites in the family Plasmodidae, genus *Plasmodium*. Twenty-three species have been described in nonhuman primates. Malaria parasites are classified based on their natural host (human, ape, monkey), on the morphology of the parasite, or on the type of cyclic fever they produce. Thus quotidian malaria have a 24hr cycle, tertian malaria have a 48hr cycle and quartan malaria have a 72 hour cycle (Figure 1, Table 1). This is determined by the time the organism parasitizes the hosts erythrocyte (red blood cell)

HINT: Record the animal's temperature every 12 hours, to indicate type of cyclic fever, and thus type of Plasmodium.

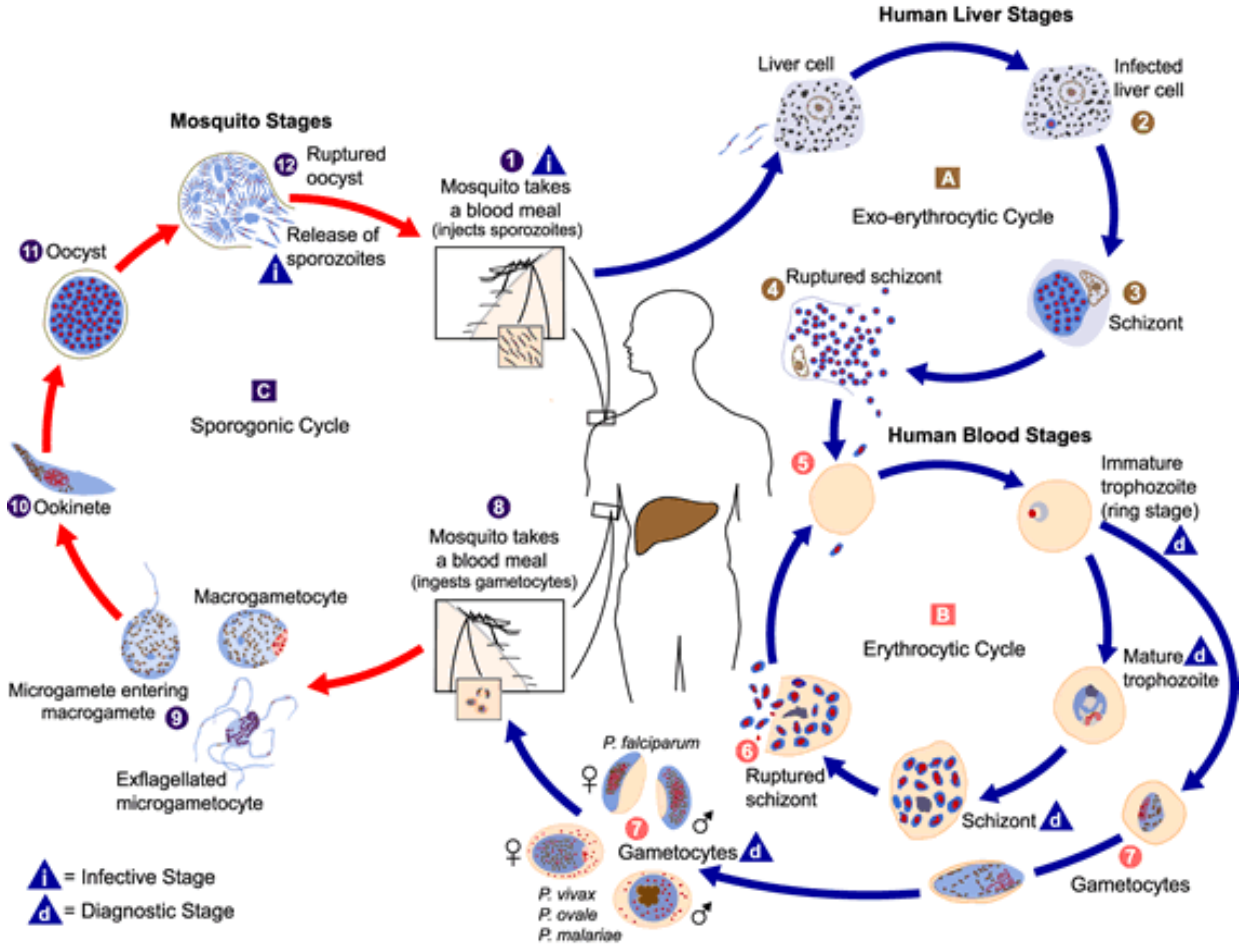


Figure 1. Malarial life cycle (human).

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release merozoites 4. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 6. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7.

Blood stage parasites are responsible for the clinical manifestations of the disease.
The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle 1.

Natural Host (Common)	**Natural Host (Scientific)**	***Plasmodium* spp.**	**Blood cycle**
Human	*Homo sapiens*	*P.falciparum, P.vivax, P.malariae*	72 hr (quartan), except vivax which is 48 hr (tertiary)
Chimpanzee	*Pan troglodytes*	*P.rodhaini* (similar to *P.malariae* and can readily be transmitted to people)	72hr Quartan
		P. reichenowi (similar to *P.falciparum* but **cannot** be transmitted to humans)	72hr Quartan
		P. schwetzi (similar to *P.vivax* and has been transmitted to humans)	48hr tertiary
Gorilla	*Gorilla gorilla*	*P.rodhaini* (similar to *P.malariae* and can readily be transmitted to people)	72hr Quartan
		P. reichenowi (similar to *P.falciparum*) but **cannot** be transmitted to humans	72hr Quartan
		P. schwetzi (similar to *P.vivax* and has been transmitted to humans)	48hr tertiary
Drill	*Mandrillus leucophaeus*	*P.gonderi*	48hr tertiary
Black Mangabey	*Cercocebus aterrimus*	*P.gonderi*	48hr tertiary
Sooty Mangabey	*Cercocebus atys*	*P.gonderi*	48hr tertiary
Agile Mangabey	*Cercocebus galeritus agilus*	*P.gonderi*	48hr tertiary

Table1. Plasmodium Species of African Primates

Thus most monkey malaria parasites exhibit tertian periodicity, completing one asexual cycle every 48 hours, and most ape malaria parasites exhibit quartan periodicity, completing one cycle every 72 hours.

Anecdotal evidence exists of malaria in guenons (Pruess' guenon) and other African monkey species, but this has not been published.

Hint: These natural infections in non human primates are unlikely to induce disease. Infection with the human strains is more likely to result in clinical disease.

The primate malarias are generally thought to have arisen from endocellular, coccidian parasites in the intestinal tract of reptiles, birds, and amphibians. The earliest primates probably carried the ancestors of present-day malaria parasites when they began to evolve in the Lower Tertiary period. The putative ancestor of present-day primate malaria parasites is Hepatocystis, a ubiquitous parasite of African monkeys and apes. This is a well-adapted, relatively benign parasite, which produces only gametocytes in the circulation. The liver stages are macrocopic and were undoubtedly the first liver stage of primate malaria seen by man, as hunters and cooks prepared monkeys for the table.

It is interesting to note that the malaria parasites of **New World monkeys**, however, are thought to have come from Europe and Africa during the slave trade. There is little evidence of malaria in the pre-Columbian New World, and the two species of monkey malaria in the New World, *P. brasilianum* and *P. simium*, are closely related molecularly, and possibly identical, to the human malaria parasites, *P. malariae* and *P. vivax*. ? Is it worth mentioning P.knowlesi from Indonesia as this is now known to infect humans as well as macaque monkeys (24 hr schizogony)

DIAGNOSIS

Equipment needs

- Accurate thermometer
- Anaesthetic and Blood collection equipment
- A good light microscope with a 100x lens for oil immersion
- Good quality glass slides
- A heat box to protect all equipment form moisture
- Field's stain (Giemsa stain, or ability to make it)

Malaria should be a major differential diagnosis in any primate in Africa with an unexplained fever. Blood should always be collected in these circumstances as part of the diagnostic workup. Blood samples can be taken by earstick or tailstick using disposable lancets. It is helpful to shave the tip of the tail or ear before taking a drop of blood in a capillary tube. The remainder of this section will concentrate on the microscopic diagnosis of malaria.

Hints on slides

USE NEW SLIDES each time or acid washed grease free slides. You can use 1N HCL/H2SO4. It is very difficult to get a uniform thickness of smear on greasy slides and so distribution of white blood cells is not uniform. This leads to inaccurate differential count. Staining quality is also not good on greasy slides. Smears should be dried quickly to prevent red blood cell (RBC) distortion. In rainy season slides should be kept in an incubator, or at least in a box with a light to keep them dry (or the addition of a bag of silica gel). If slides are not completely dry, water particles are seen after staining which may be falsely interpreted as hypochromia (Figure 2). At the same time, inclusion bodies are missed e.g., malarial parasites, Howell-Jolly bodies etc.

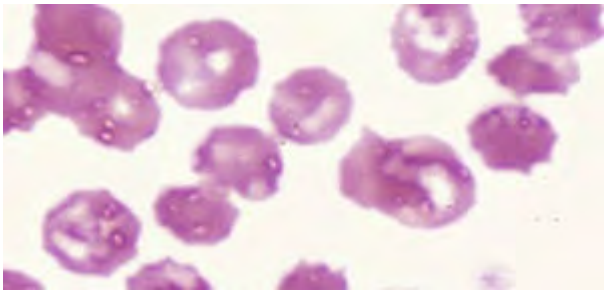


Figure 2. Water particles in poorly dried smears

Preparing Blood Smears

If you are using venous blood, blood smears **should be prepared as soon as possible after collection (EDTA is a better anticoagulant to use than heparin as the latter may affect the quality/colour of the stain)** (delay can result in changes in parasite morphology and staining characteristics). The thin smear should be fixed as soon as it is dry; the thick smear must be allowed to dry thoroughly before staining.
Thick smears should be examined for at least 5 minutes, corresponding to 100 oil immersion fields; thin films must be examined for 15 minutes before a monkey can be declared negative for malaria parasites. In doubtful cases, repeated blood smears can be made daily, taking into consideration that anesthetising a monkey to make a blood smear may be more stressful than the infection.

Thick films

Thick blood films consist of a layer of red blood cells piled thickly and irregularly to produce approximately a x30 concentration than in an equal area of a thin blood film. Due to this concentration effect thick films increase the sensitivity of parasite detection but are not usually adequate for species identification for which a thin film is usually required. Thick films should be made quickly, because undue delay may lead to fibrin formation or promote the auto-agglutination of red cells in anaemic blood. A thick blood film is not fixed and the aqueous stains

used simultaneously lyse the red blood cells, a feature essential for later examination.

Prepare at least 2 films per patient!

- Place a small drop (around 5ul)of blood in the centre of the pre-cleaned slide.
- Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it is the area of around 2.0 cm^2.
- A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.
- Keep the slides horizontal, *on an even surface,* and allow the smears to dry thoroughly (protect from dust and insects!). Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made with anticoagulated blood. At room temperature and high humidity drying can take several hours. Ideally leave films to dry for 15 mins at room temperature (20C) then for 5 minutes at around 35C (incubator). Drying may be accelerated by placing the nearly dry slide upright on a hotplate using a heat setting that one can comfortably touch OR by using a fan or hair dryer (use cool & not too fast a setting). Protect thick smears from hot environments to prevent heat-fixing the smear.

Thin films

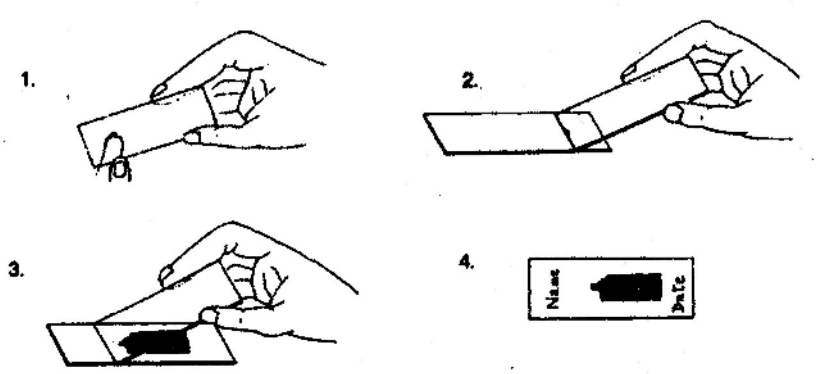
Thin films consist of blood spread in a layer such that the thickness decreases progressively toward the feathered edge, ideally the smear should be thin enough so that a large area of the slide should be a monolayer with the cells not touching one another.

Prepare at least 2 films per patient!

- Place a small drop of blood on the pre-cleaned, labelled slide, near its frosted end
- Bring another slide backwards into the drop of blood at an angle of about 30-45° (steeper for anaemic blood) allowing the drop to spread along the contact line of the 2 slides then quickly push the spreader (upper) slide forwards using a steady movement to produce a film which has 2 straight sides, does not reach the end of the slide and has a feathered edge.
- A good thin film is achieved by using the correct amount of blood and spreading technique
- Allow the thin smears to air dry – this may be hastened by *blowing gently on the film.* (Thin films should dry much faster than thick films, if not they are probably too thick.)
- If unable to stain immediately the thin films should be fixed by dipping them in absolute methanol and air drying upright.

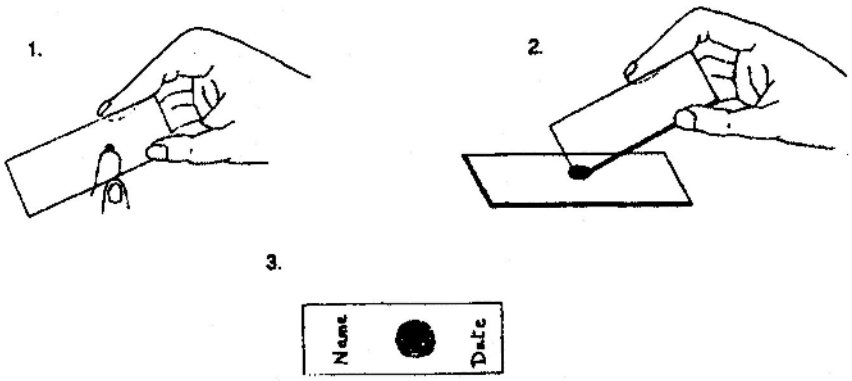
HINT: Under field conditions, where slides are scarce, you can prepare both a thick and a thin smear on the same slide. This works adequately if one makes sure that of the two smears, only the thin smear is fixed.

METHOD OF PREPARING A THIN BLOOD FILM



1. Transfer a small drop of blood to one end of a clean microscope slide.
2. Allow the blood to run along the back edge of another slide, the 'spreading slide'.
3. Push the spreading slide along the blood slide, gently, evenly and quickly
4. Label the blood slide with the name (or identification) of the patient and the date.

METHOD OF PREPARING A THICK BLOOD FILM



1. Transfer two or three drops of blood to the centre of a clean microscope slide.
2. Spread the blood rapidly and evenly into an area about 1.5cm in diameter, using a needle or the corner of another slide.
3. Label the slide

STAINING BLOOD FILMS

Stain only one set of smears, and leave the duplicates unstained. The latter will prove useful if a problem occurs during the staining and/or if you wish later to send the smears to a reference laboratory. All Romanowsky stains (Giemsa, Leishman's, Field's, Wright-Giemsa) will stain blood parasites but some give better results than others.

Wright (Wright-Giemsa) stain

Used in hematology, this stain is not optimal for blood parasites. It can be used if rapid results are needed, but should be followed up when possible with a confirmatory Giemsa stain, so that Schüffner's dots can be demonstrated.

Field's stain

This Romanowsky stain may be used for the detection and identification of blood parasites . It has the advantage of speed- a thick film is stained in 18 seconds and a thin in 24 seconds!

Field's stain may be purchased as a commercial "compound" powder, as a liquid ready to use stain or alternatively the constituent dyes may be purchased to prepare one's own stain. The advantage of the commercial compound powder is that it only requires the addition of the correct quantity of distilled (or filtered/boiled/cooled water). If the stain is prepared from base powders then these must be dissolved in phosphate buffer.

A Methylene Blue 0.4g

Azur B (I) 0.25g

250ml phosphate buffer

B Eosin (yellowish, water sol,)0.5g

250 ml phosphate buffer

STOCK Phosphate buffer:

1. Di sodium hydrogen phosphate Na2HPO4 (anhydrous) 28.39 g
2. Sodium dihydrogen phosphate NaH2PO4.H2O 27.6g

Dissolve each salt TO 1 Litre of distilled water (place chemicals in 1L flask and add water slowly to dissolve, make up to 1L mark, these stock buffers will last for approximately 6 months. For use :

Stock 1 : 360ml, **Stock 2** :140ml, plus 500ml distilled water makes 1L at pH7.2

Field's A: Add eosin to 250ml of buffer, mix well, leave for 24hrs then filter. Ready to use no further diluting.

Field's B: Grind Azur B in a mortar with a few ml of buffer taken from the remaining 250ml. Place methylene blue in a suitable container and add about 200ml of the phosphate buffer, add the liquefied Azur stain to the methylene blue mix, rinse mortar with remainder of buffer and add to stain. Mix well, leave overnight and filter next day (Whatman's no. 1 paper).

Using commercial compound powder (tcsbiosciences.co.uk)

Add 2g of Fields A (HD1410) to 80ml of DW, mix. Add 1 g of Fields B compound (Cat. No. HD 1415) to 80ml of distilled water, mix. The 80ml volume will just fit into standard Coplin jars stain and is ready for use. These jars of stain may last for 1 month or more depending upon how frequently the stain is used.

Staining Method Thick Films

Lysis and staining of a thick blood film occur simultaneously.

1. Dip thick film in solution A for 5 seconds, drain. (avoiding agitation of the stain, because this will disperse bacteria concentrated toward the bottom of the container, and cause them to adhere to the blood film).
2. Wash gently in a jar of water for 5 seconds, drain.
3. Dip in solution B for 3 seconds, drain.
4. Wash again in the jar of water for 5 seconds.
5. Stand the slide nearly upright to allow the haemoglobin to drain out while the slide dries.

The times given are approximate, and vary with batches and age of stain and the thickness of the films. The film is usually of varying thickness, and different parts take up the stain at different rates. The optimum staining of parasites, usually near the edges of the film, occurs where the nuclei of the leucocytes are stained a rich purple colour.

An oxidation scum will appear on the surface if the stain is used infrequently, but this scum can be removed by drawing filter paper across the surface. Ideally, the stains should be filtered daily and renewed weekly in laboratories in warm climates.

Reverse Field's stain for thin films

1. Fixed thin film by dipping in methanol (or industrial methylated spirit) for 8 seconds (8 "dips"- lifting slide out of stain after each dip)
2. Dip into Field's B (eosin) for 8 seconds.
3. Wash for 8 dips in a jar of tap water, agitating well.
4. Dip into Field's A for 8 dips
5. Wash for 8 seconds in a second jar of tap water .
6. Dry upright.

Giemsa stain

Recommended for detection and identification of blood parasites. I've provided the formula below for reference, but commercial preparations of this stain are available. However, the advantage of preparing your own is that the formula gives more consistent results, and doesn't expire.

Stock 100× Giemsa Buffer preparation	0.67 M
Na_2HPO_4	59.24 g
NaH_2PO_4H2O	36.38 g
Deionized water	1000.00 ml

Autoclave or filter-sterilize (0.2 μm pore). Sterile buffer is stable at room temperature for one year.

Working Giemsa Buffer preparation	0.0067M, pH 7.2
Stock Giemsa Buffer	10.0 ml
Deionized water	990.0 ml

Check pH before use. Should be 7.2. Stable at room temperature for one month.

Triton X-100 5% preparation	
Deionized water (warmed to 56°C)	95.0 ml
Triton X-100	5.0 ml

Prewarm the deionized water and slowly add the Triton X-100, swirling to mix.

Stock Giemsa stain preparation

Glass beads, 3.0 mm	30.0 ml
Absolute methanol, acetone-free	270.0 ml
Giemsa stain powder (certified)	3.0 g
Glycerol	140.0 ml

- Put into a 500 ml brown bottle the glass beads and the other ingredients, in the order listed. Screw cap tightly. Use glassware that is clean and dry
- Place the bottles at an angle on a shaker; shake moderately for 30 to 60 minutes daily, for at least 14 days
- Kept tightly stoppered and free of moisture, stock Giemsa stain is stable at room temperature indefinitely (stock stain improves with age)
- Just before use, shake the bottle. Filter a small amount of this stock stain through Whatman #1 filter paper into a test tube. Pipet from this tube to prepare the working Giemsa stain

Working Giemsa stain (2.5%): make fresh for each batch of smears

Working Giemsa buffer	39 ml
Giemsa Stain Stock	1 ml
5% Triton X-100	2 drops

STAINING PROCEDURE

- Prepare fresh working Giemsa stain in a staining jar, according to the directions above. (The 40 ml fills adequately a standing Coplin jar; for other size jars, adapt volume but do not change proportions).
- Pour 40 ml of working Giemsa buffer into a second staining jar. Add 2 drops of Triton X-100. Adapt volume to jar size.
- The thin smear is fixed in absolute methanol for 2 minutes; the thick smear is left unfixed
- Place slides into the working Giemsa stain (2.5%) for 45-60 minutes at a pH of 7.25.
- Remove thin smear slides and rinse by dipping 3-4 times in the Giemsa buffer. Thick smears should be left in buffer for 5 minutes.
- Dry the slides upright in a rack.

If using Giemsa stain, Giemsa improved staining solution (Gurr's R66) works well for malaria parasites. Using R66 at a dilution of 1:10 in buffered water pH7.2 staining time is about 15 minutes. Slides should be rinsed in buffered water for optimum staining.The main drawbacks of using Giemsa is that the stain must be diluted daily before use and any excess stain discarded at the end of the day.

An easy way of making buffered water is by using commercial buffer tablets : 1 tablet to 1 litre water (VWR- cat no. 1.09468.0100, Merck buffer tablets ph7.2, pack 100 tabs)

Over staining (figure 3) can leave stain particles on slides which may be sometimes mistaken as platelets.

Figure 3. Stain particles in overstained slides

MICROSCOPIC EXAMINATION OF FILMS

Examining thick films

Since the erythrocytes have been lysed and the parasites are more concentrated, the thick smear should be used to screen for parasites and for detecting mixed infections.

- First examine the entire smear at a low magnification (10× or 20× objective lens), to detect large parasites such as microfilaria. If working in an area where trypanosomes may be present these may be seen more easily using a x40 objective).
- Then examine the smear using the 100× oil immersion objective lens. If using Field's stain select an area that where the nuclei of the white blood cells (WBCs) are stained purple- in this area parasite cytoplasm will stain blue and nuclear material (chromatin) a dark red. Ideally examine an area with sufficient number of WBC's ,field (10-20 WBCs/field).
- If parasites are seen , make a note of the stages seen (mainly rings,etc) and look for the presence of Schuffner's dots (seen as a halo of pink around the parasites) to help make a tentative species determination. Examine the thin smear to determine species of malaria. With practice it may be possible to determine species from the thick film.
- Determination of "No Parasites Found" (NPF): For malaria diagnosis, the World Health Organisation recommends that at least 100 fields, each containing approximately 20 WBCs, be screened before calling a thick smear negative. Assuming an average WBC count of 8,000 per microliter of blood, this gives a threshold of sensitivity of 4 parasites per microliter of blood. In non-immune human patients, symptomatic malaria can occur at lower parasite densities, and it is normal to examine 200 oil immersion fields on 2 different films before declaring no parasites found.

Examining thin films

Thin films are useful for species identification of parasites already detected on thick smears, screening for parasites if adequate thick smears are not available, and a rapid screen while the thick smear is still drying. If NO thick film is available it is recommended that the thin film is examined for 30 minutes before declaring no parasites found .

Some parasitised red blood cells roll to the edges and are carried to the tail of the film, so that these parts should be examined for malaria parasites, in addition to those areas of the film where the red cells are just separated. Try to avoid parts of the film where the red blood cells are piled on top of each other. As haemoglobin is retained during staining, malaria parasites appear framed by the red blood cells.

- **Screen at low magnification (10× or 20× objective lens) if this has not been done on the thick smears.**
- **Carefully examine the smear using the 100× oil immersion objective lens. .**

Quantifying parasites

In some cases (especially malaria) quantification of parasites yields clinically useful information as the malaria parasites can be quantified against blood elements such as RBCs or WBCs. . Only asexual blood stage parasites should be counted as gametocytes of all species may persist following treatment, gametocytes will die in the host within a month and cannot continue their cycle unless they are sucked up by an anopheline mosquito.

Only asexual blood stage parasites should be counted as gametocytes of all species may persist following treatment, gametocytes will die in the host within a month and cannot continue the ir cycle unless they are sucked up by an anopheline mosquito.

Parasite numbers fluctuate daily for a variety of reasons. A series of counts over several days will usually indicate a general decline, stabilisation or increase. Estimates of parasitaemia are important in monitoring the response of patients to treatment. However, as parasite numbers generally rise for about 12-18 hours after the beginning of successful therapy, only a continuing rise beyond this period is of concern.

Percentage of parasitised red cells using thin blood film

1. The thin film should be a well-prepared and stained monolayer. Using the x 100 (oil immersion) objective find a field that contains an infected erythrocyte.
2. Reduce the field size either by using an Ehrlich's eye piece until the field of vision contains about 50 erythrocytes or by using an eyepiece graticule to “section” the field of view.
3. Using 2 hand tally counters, count the total number of cells in the field on one counter; use the other to count the number of erythrocytes infected with **asexual** stages of parasites.
4. Count a minimum of 1,000 erythrocytes and express the parasitaemia as a percentage. % parasitemia = (parasitized RBCs/total RBCs) × 100
5. Repeat the process in another area of the film and take the mean of the two results

NB If the parasitaemia is <1% a more accurate estimation of parasitaemia will be obtained by counting parasites on a thick blood film. Table 2 is an *estimate* of parasite numbers if the same microscope is used each time for examining blood films.

Parasites observed	Percentage of red cells parasitized
10-20 per field	1
1-2 per field	0.1

1-2 per 10 fields	0.01
1-2 per 100 fields	0.001
1-2 per 1000 fields	0.0001

Table 2. Estimates of parasitaemia from thick film using a x 100 oil immersion objective and x 10 eyepieces. Confidence limits of low counts are fairly wide.

Number of parasites/µl blood using thick blood film

Malaria parasites are counted in relation to the patients' total white cell count. If the total white cell count is unavailable it is assumed to be 8,000 WBC/ µl .

1. The thick film is examined using the x100 oil immersion objective and leucocytes are counted on one tally counter until 100 are recorded.
2. At the same time, using another tally counter, the total number of **asexual** stages of malaria parasites seen are recorded.
3. This process is repeated twice, in other areas of the film, until a total of 300 WBC's have been recorded. Calculate the mean number of parasites per 100 WBC's.
4. The number of **parasites/µl** is calculated using the formula:

$$\frac{\text{No.parasites x WBC/µl (or 8,000)}}{100}$$

An alternative (and less accurate) method for estimating the number of parasites in **thick films** is as follows:

1-10	per 100 high power fields	+
11-100	per 100 high power fields	++
1-10	in every high power field	+++
more than 10	in every high power field	++++

Results in % parasitized RBCs and parasites per microliter blood can be interconverted if the WBC and RBC counts are known (thus ALWAYS do a Complete Blood Count (CBC)), or otherwise (less desirably) by assuming 8,000 WBCs and 4,000,000 RBCs per microliter blood.

Dipstick tests for circulating antigen (HRP2, pLDH) may give positive results for *P. cynomolgi*, *P. coatneyi*, and *P. knowlesi, HRP2 type tests may remain positive for up to 2 weeks following successful treatment, pLDH type tests will only detect LIVE parasites.* Other non-human primate malarias have not been tested. PCR is not generally useful in a clinical setting unless one is interested in specific molecular sequences. It is not more sensitive than a well-made thick smear and available primer sequences are limited to *Plasmodium* species used in malaria model work, e.g. *P. cynomolgi*, *P. knowlesi*, and *P. coatneyi*.

HINT: A cautionary note on Babesia

Other Apicomplexan parasites that may appear in blood smears and confused with malaria parasites, are the piroplasms (family *Babesidae*). *Entopolyploides macaci* is often reported in rhesus macaques (*Macaca mulatta*), African monkeys (*Cercopithecus*), and baboons (*Papio*) but will infect a variety of non-human primates. The organisms are smaller than *Plasmodium*, markedly pleomorphic, and produce no pigment. The infections are usually benign even in splenectomized animals, and do not respond to standard antimalarial therapy. Unlike *Plasmodium*, they are tick-borne.

MICROSCOPIC IDENTIFICATION.

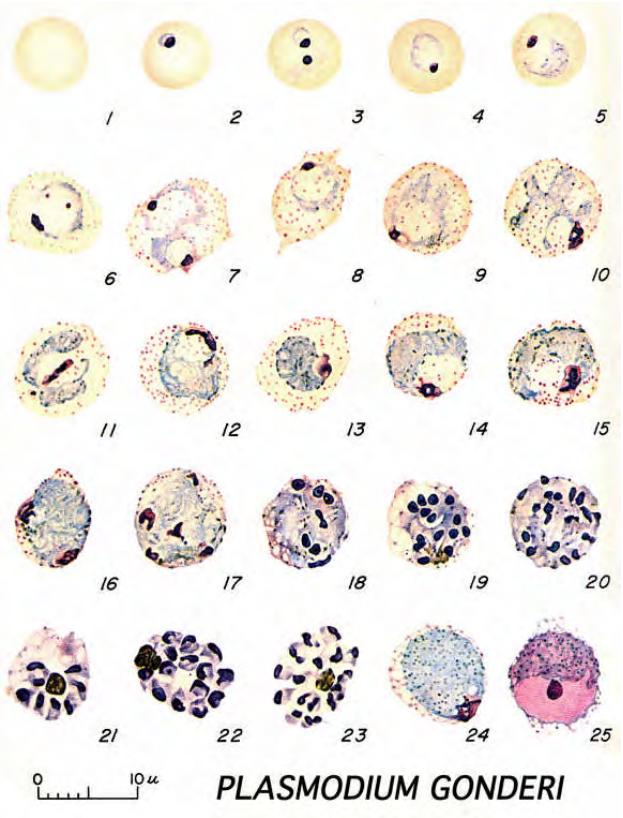


Figure 4. Plasmodium stages in drills and mangabeys

1: Normal red blood cell. 2-4: Young trophozoites. 5-11: Growing trophozoites. 12-15: Mature trophozoites. 16-20: Developing schizonts. 21-23: Mature schizonts. 24: mature macrogametocyte 25: Mature microgametocyte.

Cycle in blood - The asexual blood cycle occupies 48 hours. Merozoites prefer to invade reticulocytes and the developing ring forms are often 2 - 4 per red cell. Schüffner's stippling appears in the cytoplasm with further growth of the parasite and the host cell becomes enlarged and distorted. Mature trophozoites show a deep blue staining cytoplasm, a large irregular red staining nucleus, and pigment in small aggregates. Pigment in the developing schizont is more condensed and the stippling is prominent. Older schizonts fill the red cell and show purple cytoplasm with reddish nuclei on the periphery of the parasite. The mature schizont may not fill the host cell and may contain 12 - 20 merozoites with an average of 16. The cytoplasm of the host cell is hypochromic almost to the point of being inapparent so that the schizont may appear free. Microgametocytes stain a light purplish pink, with dark pigment granules scattered in the cytoplasm. The macrogametocyte stains deep blue with scattered pigment.

Course of Infection - In sporozoite-induced infections, peak parasite counts are on the order of 190,000/mm^3 at day 10 of patent parasitemia. The counts then decline to a low level (about 350/mm^3). Animals do not generally require chemotherapy for survival.

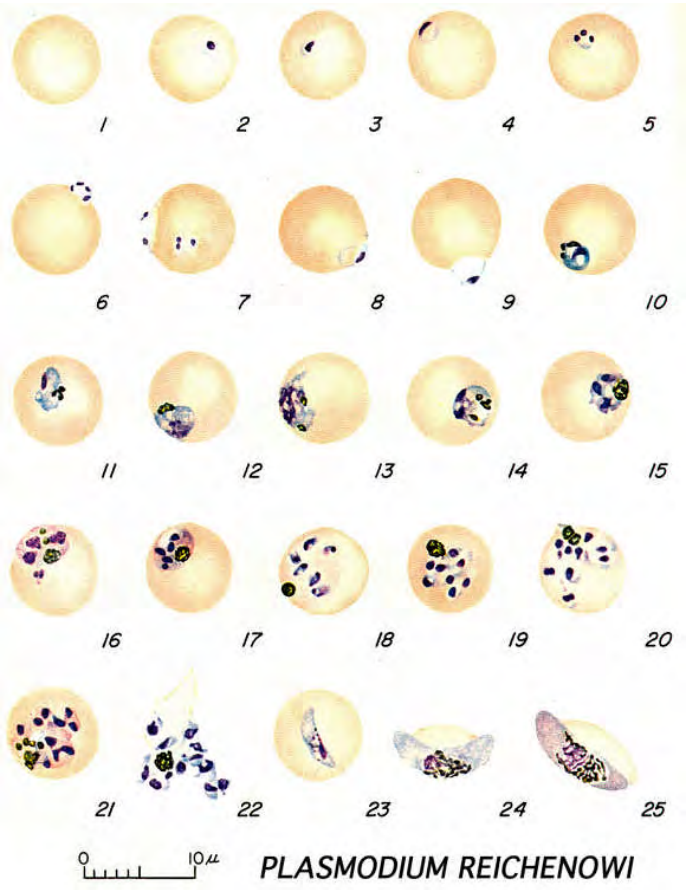


Figure 5. Plasmodium stages: Chimpanzees and Gorillas

Plasmodium reichenowi, cycle in blood. 1: normal red cell. 2 - 9: Young trophozoites. 10 - 13: Growing and mature trophozoites. 14 - 17: Developing schizonts. 18 - 22: mature schizonts. 23 , 24: Young adult and mature macrogametocytes. 25: Mature microgametocyte.

Cycle in blood - The asexual blood cycle occupies 48 hours. The parasite closely resembles *P. falciparum* of man, with crescent-shaped gametocytes and usually only ring stages and gametocytes appearing in the peripheral circulation. The youngest parasites are small rings with a prominent nucleus. A consistent feature is the presence of marginal forms with single or double nuclei; accolé forms are common. The mature schizonts contain 10 - 12 merozoites, but may not be seen in the peripheral circulation, unless the animal is splenectomized. The mature macrogametocyte is crescent-shaped and somewhat slender with pale blue cytoplasm and a red-staining nucleus. The microgametocyte is more robust with bluish-red cytoplasm and a diffuse red-staining nucleus.

Course of Infection - **Little is known** about the early course of naturally-occurring infections. There is only meagre information about naturally-infected and blood-inoculated captive animals.

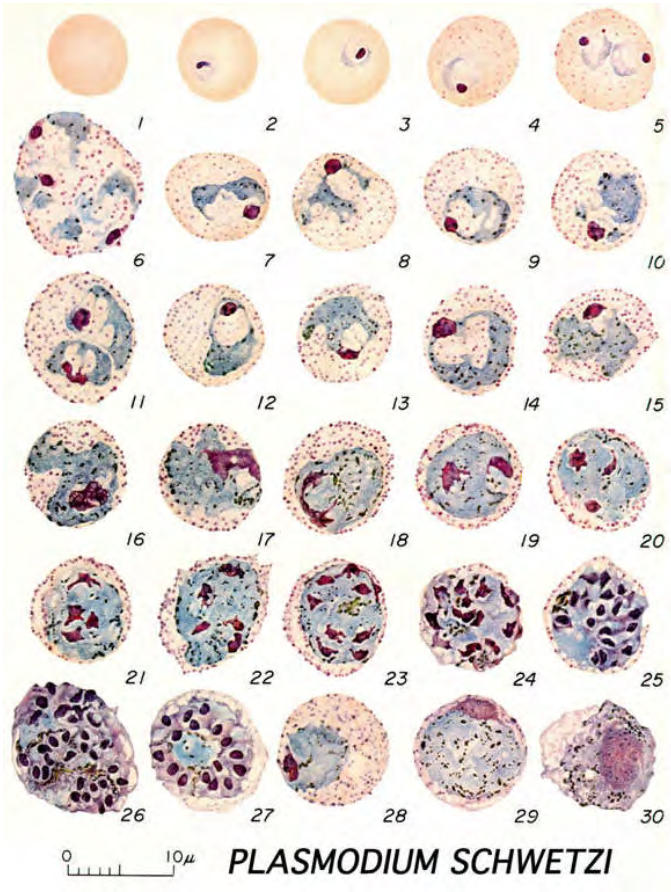


Figure 6. Plasmodium stages: Chimpanzees and gorillas

Plasmodium schwetzi, cycle in blood. 1: Normal red cell. 2,3: Young trophozoite. 4 - 14: Growing trophozoites, showing double and triple infections. 15 - 18: Older and mature trophozoites. 19 - 24: Developing schizonts. 25 - 27. Nearly mature and mature schizonts. 28,29: Half-grown and mature macrogametocytes. 30: Mature microgametocyte.

Cycle in blood - The parasite has a 48 hour asexual cycle in the chimpanzee. The earliest ring forms are relatively compact with a dark, round to oval nucleus. Growing parasites enlarge the host cell and stippling is abundant in the older trophozoite stage. The mature schizont has from 12 - 16 distinct nuclear masses and the red cell may be distinctly oval shaped. The macrogametocyte stains uniformly blue with a peripheral wine-red nucleus. The microgametocyte is brightly colored with a reddish-purple cytoplasm and a large, diffuse wine-red nucleus. The pigment is coarse, black to greenish black and the cytoplasmic edge of the parasite is crenated or lace-like.

Course of Infection - *P. schwetzi* appears to invoke **no clinical signs**, even in young chimps, nor in splenectomized older animals with high parasitemias. It generally occurs as a mixed infection with *P. reichenowi* and *P. rodhaini*.

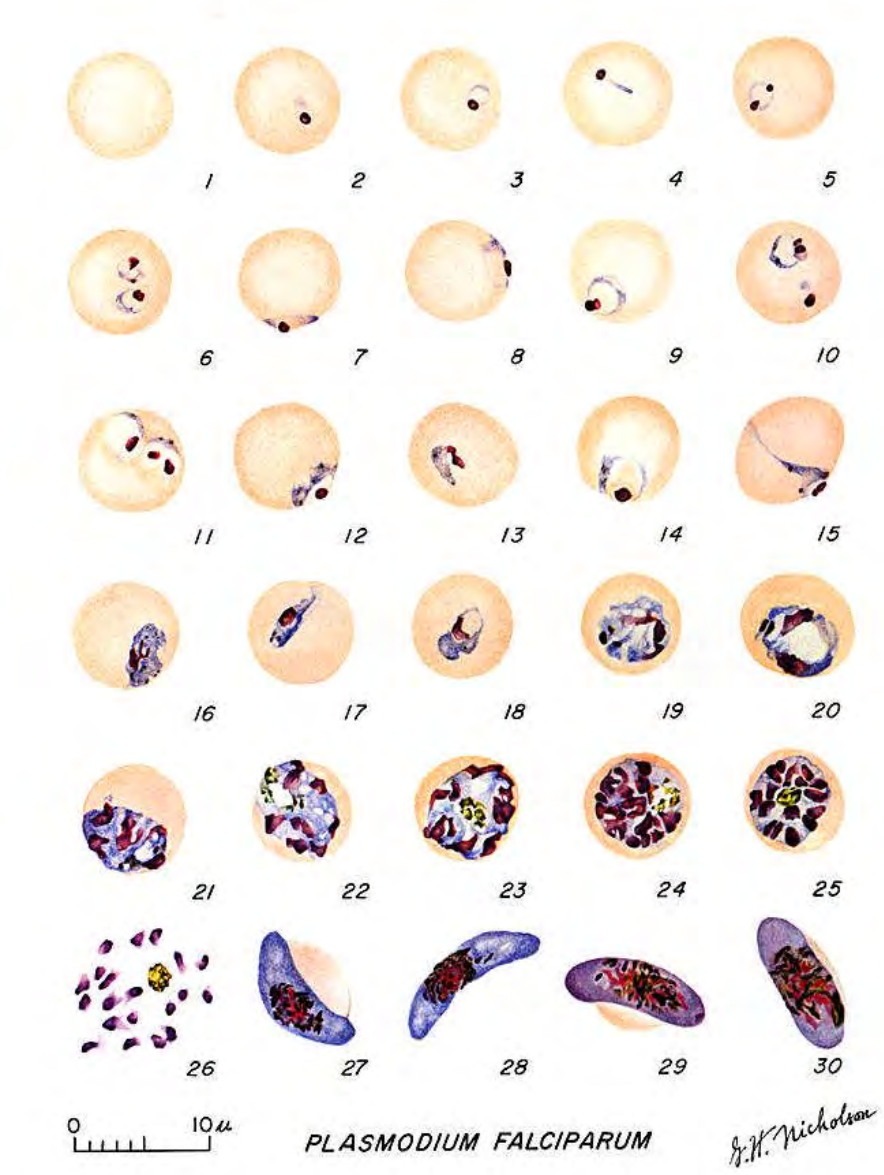


Figure 7. Malaria in humans – please refer to general life cycle

MICROSCOPIC EVALUATION

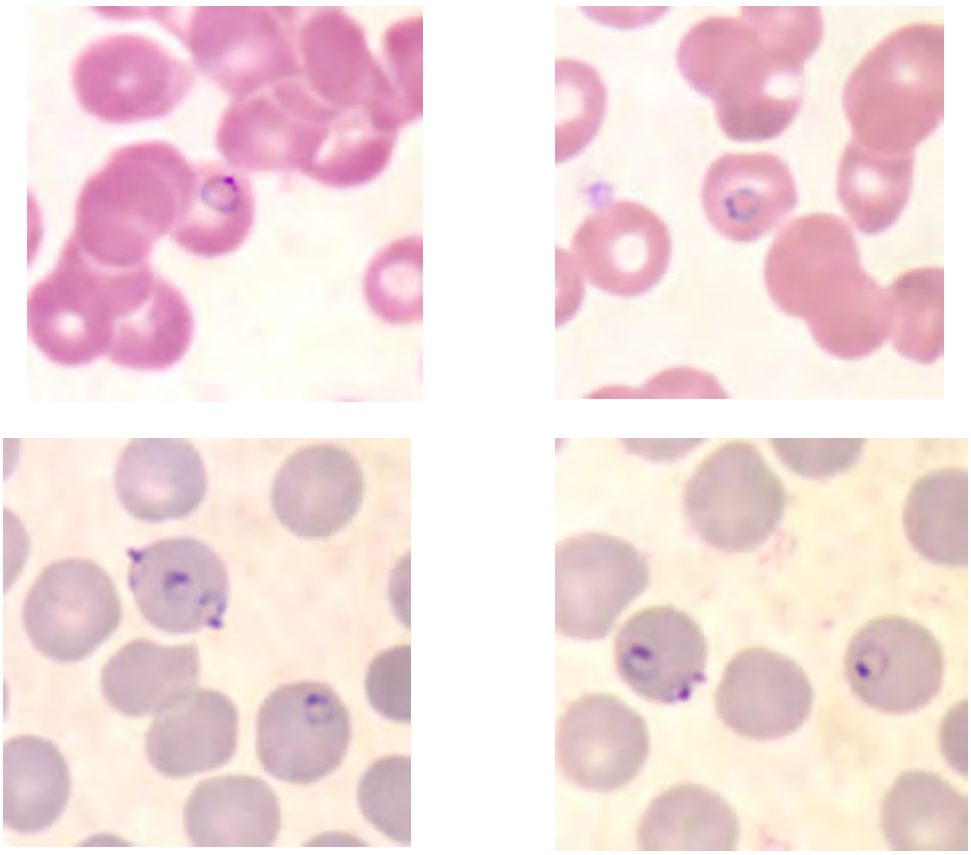


Figure 8-11 Ring form (young trophozoites) – thin smears.

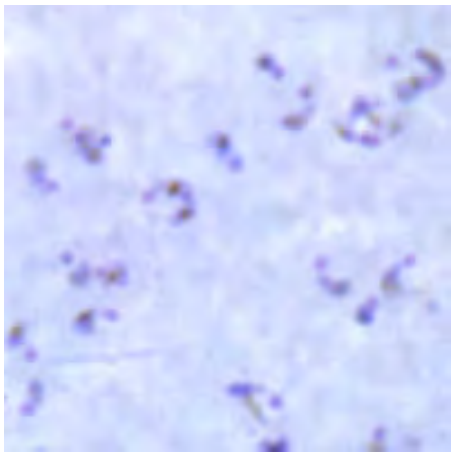


Figure 12 Ring Form (young trophozoites) – Thick smear. F

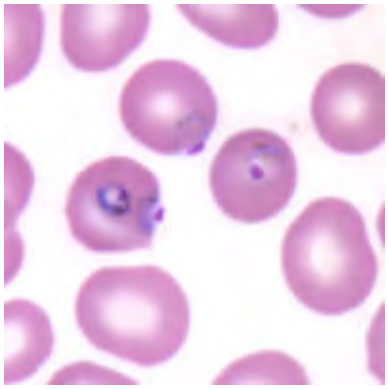
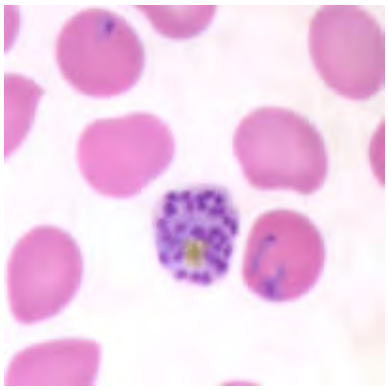


Figure 13. Mature trophozoites



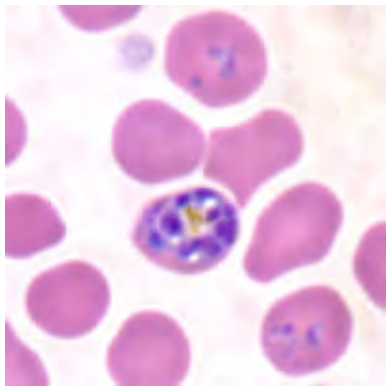
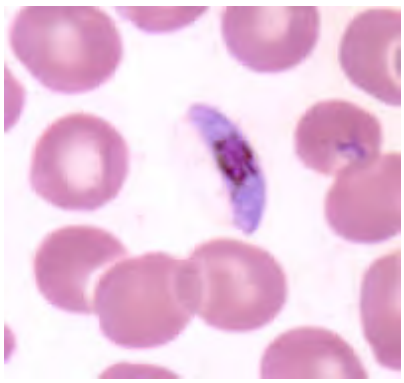


Figure 14 and 15. Schizonts.



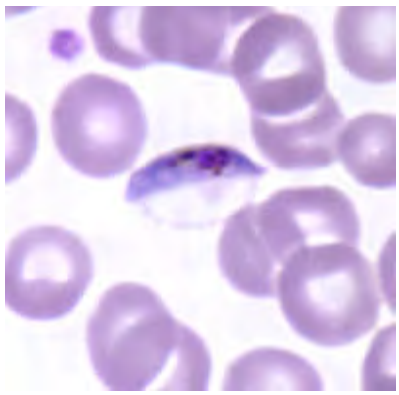
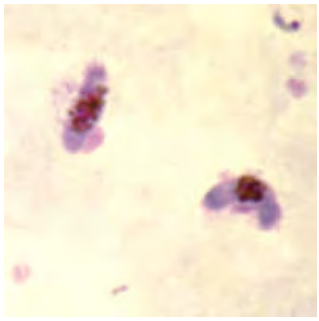


Figure 16 and 17. Gametocytes – thin smears.



Gametocytes – Thick smears. Figure 19

PATHOLOGY

Most primate malarias and infections with piroplasms are **subclinical, unless the animal is immunosuppressed or splenectomized** (had the spleen removed for some other disease). Experimental infections of rhesus macaques with *P. knowlesi*, *P. coatneyi*, and less often *P. cynomolgi*, may be characterized by jaundice, anorexia, listlessness, fever, anemia and splenomegaly in spleen-intact animals. Clinical signs of chills and fever are in response to toxins (*Plasmodium* GPI) exposed during the release of merozoites from the red cell. Pregnant animals may experience more severe anemia, which will have a measurable impact on the health of the fetus.

If the animal succumbs to infection, on post mortem, grossly the lungs, liver, and spleen are grey and the blood thin. Histologically, tissue macrophages are filled with malaria pigment and there are hemosiderosis and parasitized RBC's. Intravascular clotting with thrombi and parasitized erythrocytes is common. Often there is pulmonary and cerebral oedema.

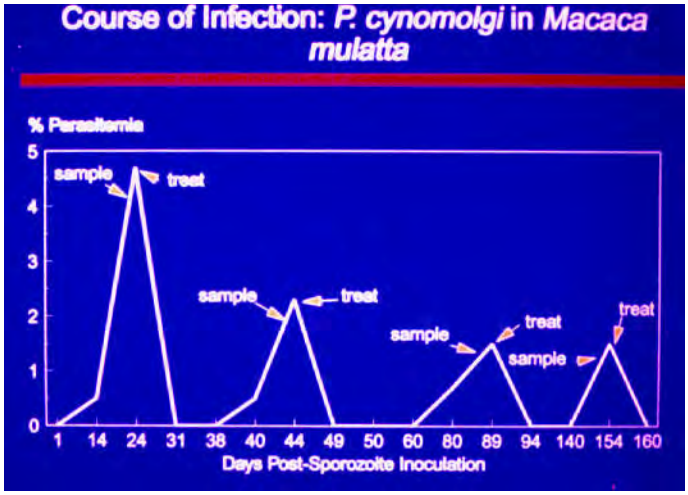
TREATMENT

Most primate malarias can be treated with chloroquine at a dosage of 7 mg/kg base for 5 days (total = 35 mg/kg). This can be given as an IM injection or per os via nasogastric tube. The bitter taste of 4-aminoquinolines precludes putting it in food. Chloroquine is effective against the circulating trophozoite (feeding) stage of the parasite but will not affect hepatic stages nor circulating gametocytes. Mefloquine (Larium) can also be used, especially if an isolate is suspected to be chloroquine-resistant, at a dose of 20 mg/kg, one dose, per os. Hepatic stages of *Plasmodium* require treatment with an 8-aminoquinoline (primaquine) at a dose of 3 mg base/kg body weight per os for 7 days. This may be necessary in sporozoite-induced (i.e. natural) infections with *P. cynomolgi*, *P. simiovale* and *P. fieldi* where malarial relapse is a consideration.

Because of increased toxicity when given together, chloroqine and primaquine should be given separately. Treatment is only necessary if reinfection occurs (ie. In endemic malarial areas).

More information is required on the possible use of new antimalarial human products in non human primates such as atovaquone and proguanil hydrochloride and artemether/lumefantrine. Widespread use of these drugs is NOT recommended, as resistance is likely to be induced more rapidly in all populations. Although research is ongoing for a malarial vaccine, this is still some years off, and potential resistance must be born in mind whatever treatment regime used.

APPENDIX



Experimental plasmodium infection and treatment regime in a macaque monkey. This regime is typical of the disease course in humans, and possibly African primates. Information on the treatment of non-human primate piroplasms has not been reported. Human infections with *Babesia microti* have been successfully treated with a combination of clindamycin and oral quinine. Adult dosage for adults is clindamycin, 1.2 g IV b.i.d. or 600 mg orally t.i.d. plus quinine 650 mg orally t.i.d., both for 7 days. Dosages for children (more similar in body weight and surface area to non-human primates) have not been reported.

	SPECIES				
	P.falciparum	*P.malariae*	*P.vivax*	*P.ovale*	*Babesia sp.*
Appearance of infected red blood cells (size and shape)	Both normal	Normal shape. Size normal or smaller	1½ to 2 times larger than normal; shape normal or oval	As for *P.vivax*, but some have irregular frayed edges	Both normal
Schüffner's dots (eosinophilic stippling)	None (but occasionally comma-like red dots present = Maurer's dots	None	Present in all stages, except early ring form	As for *P.vivax*	None
Red cells with multiple parasites/cell	Common	Rare	Occasional	As for *P.vivax*	Common
Stages present in peripheral blood	Rings and gametocytes; occasionally schizonts	All stages	All stages	As for *P.vivax*	Only rings and rare pear-shaped forms (`Maltese cross'). No gametocytes
Ring form (young trophozoite)	Delicate, small ring; scanty cytoplasm; sometimes at the edge of red cell (accolé' form)	Ring 1/3 diameter of cell; heavy chromatin dot; vacuole sometimes `filled in'	Ring 1/3 - 1/2 diameter of cell; heavy chromatin dot	As for *P.vivax*	Resembles ring of *P.falciparum.* Look for pear-shaped structure
Schizont	Occasionally in peripheral blood, 16 - 30 merozoites	6-12 merozoites in rosette; coarse pigment clump in centre	12-24 merozoites in rosette filling entire RBC; central pigment	8-12 merozoites in rosette	No schizont
Gametocyte	`Crescent' or `sausage' shape are characteristic	Round or oval; dark coarse pigment	Round or oval	Round or oval (smaller than *P.vivax*)	No gametocyte
Main criteria	Delicate ring forms and crescent shaped gametocytes are the main forms in the bloodstream. Multiple infection common. Normal RBC shape/size.	Red cell normal or slightly smaller; trophozoites compact and intensely strained; band-form suggestive; no Schüffners dots; coarse and dark pigment.	Large pale RBC; presence of Schüffners dots in cytoplasm of RBC; round gametocytes; large amoeboid trophozoite with pale pigment	Oval RBC with fimbriated edges characteristic but not always present; generally like *P.vivax*	Ring forms very similary to *P.falciparum*; presence of group of 2-4 pear-shaped bodies (`Maltese cross') is characteristic; absence of gametocytes

P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
Often numerous delicate rings	Ring forms not specifically identifiable	Ring forms not specifically identifiable	Ring forms not specifically identifiable
Excepting gametocytes, all parasites are at the same stage of development (ring/trophozoites)	All stages may be seen	All stages may be seen	All stages may be seen
Rings may be turned so appear as exclamation mark or propellers	Older trophozoites often amoeboid	Parasites solid and contain pigment	Older trophozoites solid, small and stain deeply
Gametocytes crescent shaped	Lysed RBC may appear as pink "ghost" around parasites	Lysed RBC as in *P. vivax*	Schizont and gametocyte compact
If blood is taken several hours before films are made, trophozoites may be very solid and compact, gametocytes may round up	Parasite may be surrounded by Schüffner's dots	As in *P. vivax* – Schüffner's dots	Coarse pigment granules in parasite

Appearance of malaria parasites in thick blood films

3.12 VACCINATIONS – GENERAL INFORMATION

Authors W. Boardman, E. Dubois, O. Slater. Reviewer S.Unwin

When vaccines are used, the type, batch number, expiration date and source of vaccine should be recorded in the medical records. The decision to vaccinate with any vaccine, with the possible exception of tetanus, must not be taken lightly, as this could severely disrupt a reintroduction programme. It is up to each sanctuary to thoroughly investigate disease issues that may be managed by vaccination within their sanctuary; what vaccines are being used in the local human population; and where possible, is it effective and safe in the species being investigated. Where vaccines are used, the following chapter highlights current vaccines available that may be useful, and their dosing regimes.

TETANUS

Three intramuscular doses of tetanus vaccine (each containing 40iu of tetanus toxoid = 0.5ml) are given at 2 - 3 month intervals, starting at 3 months of age. Intramuscular boosters are given after 5 years and at 10-year intervals thereafter.

POLIO

Three doses of live trivalent polio vaccine (containing live attenuated strains of poliomyelitis virus, types 1, 2 & 3) are given at 2 - 3 month intervals. This may be started at 3 months of age (or earlier if a particularly high risk exists). Oral boosters are given after 4-6 years. These vaccines have been found to be safe and effective in primate species. It is important to give the oral polio vaccine to all primates in a group at the same time. This is particularly important with the first dose of any course. Keepers that have been vaccinated during the previous 7 days should not be working with unvaccinated primates. Keepers should of course all be vaccinated up to date.

MEASLES

Two doses of MMR (Measles, mumps, rubella) are routinely recommended at 12-15 months and again at 4-6 years (or the next time the animal is handled again provided that the second dose of MMR is given at least 28 days after the first dose). The second dose is not a booster, but is given due to the fact that studies in humans show that 2-5% of individuals do not develop immunity after the first dose.
Measles vaccine may be effective if given within the first 3 days after exposure and it is recommended to vaccinate during the course of an outbreak.
MMR vaccine can be given to great apes with nursing infants with no risk to the nursing infant. MMR should not be given to any animals known to be pregnant or animals with the potential to become pregnant within 30 days of vaccination due to the theoretical risk of fetal infection. Recent research has shown that there is no link between MMR vaccination and autism in humans.

Note: Live virus vaccines can interfere with the response to tuberculin testing. Tuberculin testing can be done either on the day that the live virus vaccines are administered or 4-6 weeks later.

Reference:
www.cdc.gov/mmwr/preview
http://www.cdc.gov/mmwr/preview/mmwrhtml/00053391.htm

RABIES

If there is a high risk of rabies, it may be advisable to vaccinate - using killed vaccines only and given intramuscularly at 6 months and one year. One to three year intervals thereafter can be used depending on which vaccine is given.

TUBERCULOSIS

Do not vaccinate against tuberculosis. BCG confers variable protection in man and short-lived protection in non-human primates. Furthermore, as there is no way of distinguishing between tuberculin reactions due to BCG or natural infection, the usefulness of the tuberculin test will be lost. E.g. Should a TB +ve primate be inadvertently added to a "clean" group, vaccination would make it almost impossible to identify infected in-contact primates and therefore the degree of spread.

OTHER VACCINES

Do not use the triple vaccine known as DPT or DTP (diphtheria, tetanus and pertussis). There have been a large number of adverse reactions recorded in other primates and they are not particularly susceptible to diphtheria or pertussis.

TYPHOID: Salmonella typhii

Infection with this bacterium has only been confirmed in non human primates experimentally. Where this disease is suspected in a sanctuary, every effort must be made to confirm the diagnosis. Until that time, vaccination for this disease can't be recommended. The regime is provided here for completeness. In humans, the vaccine needs to be given one week prior to exposure to be effective. The vaccine loses effectiveness after several years. The oral vaccine is not recommended prior to six years of age. One capsule by mouth, repeated every two days for four doses with a booster required every five years is recommended. A parental vaccine is also available and can be given at two years with boosters every two years thereafter.

http://www.cdc.gov/ncidod/dbmd/diseaseinfo/TyphoidFever_g.htm#Getting%20vaccinated
Pneumococcal vaccine (Streptococcus pneumoniae). Ineffective in Non human primates.

CHIMPANZEE VACCINATIONS.

1. TETANUS TOXOID

Schedule: 0.5 ml given IM at 2, 4, 6 (or whatever is feasible), 15-18 months, 4-6 years, and then every 5 years thereafter. Check record prior to annual exam to assess vaccine status. If the chimpanzee is an adult with unknown vaccination history provide three doses with the second dose given 1-2 months after the first dose and the last dose at 6 to 12 months.

2. MEASLES VIRUS VACCINE

Schedule: Give 0.5 ml SQ at 12-15 months with a repeat dose at least 28 days after the first dose but no later than 4-6 years. Check record prior to annual exam to assess vaccine status. If the chimpanzee is an adult with unknown vaccination history provide two doses with at least a 28 day gap between the first and second doses.

Precautions: Should not be given to pregnant females, thus a pregnancy exam should be performed prior to administration. Females should not become pregnant 3 months post vaccination.

3. KILLED POLIOVIRUS VACCINE

Schedule: 0.5 ml given orally at 2, 4, and 15-18 months, and 4-6 years.
If the chimpanzee is an adult with unknown vaccination history provide three doses with the second dose given 1-2 months after the first dose and the last dose at 6 to 12 months.

4. KILLED RABIES VIRUS VACCINE

Schedule: Give 1 ml SQ or IM at 16 weeks and then every 3 years.

Table of Vaccination Recommendations

Age	Vaccine type
2 months	Tetanus Polio
4 month	Tetanus Polio
6 months	Tetanus
15-18 months	Tetanus Polio Measles
4-6 years	Tetanus Polio Measles

Every 10 years	Tetanus

(Adapted from Loomis; Update of vaccination recommendations for non-human primates; Proc AAZV, 257-260, 1990 and human recommendations from the CDC)

3.13 AFRICAN PRIMATE HANDLING AND ANAESTHESIA

S.Unwin, M. Ancrenaz, S.Mahe and W. Boardman

Introduction.

This section will provide an overview of anaesthesia in African primates including highlighting various chemical restraint regimes and suggesting methods of anaesthesia for specific species. It aims to outline what is anaesthetic best practice, indicate instances and procedures where anaesthesia may not even be necessary, and also to investigate how each anaesthetic regime works. The section is divided into three parts. Part 1 outlines general anaesthetic principles and processes, including a brief section on manual restraint. Part 2 deals with anaesthetic emergency procedures when things go wrong. Part 3, discusses anaesthetic drug properties and provides anaesthetic suggestions by species.

Chemical immobilisation of wild primates is a difficult, risky and hazardous procedure. Besides the risks related directly to the capture itself, several authors have stressed the problem of behavioural disruption of a group following an anaesthetic event, resulting for example in a change in the social status of the darted individual (Chimpanzee *Pan troglodytes*; S. Unwin personal observations). This can also manifest as an altered response to the human observer (Karesh *et al*., 1998). . Procedures for darting small arboreal primates are described by Glander *et al.* (1991), Jones & Bush (1988) and Karesh *et al*. (1998); guidelines for chemical capture of large terrestrial NHPs can be found in Sapolsky & Share (1998) and Sleeman *et al.* (2000); practical tips for chemical restraint of mammals as well as a list of the necessary equipment for field anaesthesia may be found in Osofsky & Hirsch (2000) Readers are also directed to the primate anaesthetic chapters in West, Heard and Caulkett (2007) for a thorough overview of primate anaesthesia.

Part 1. The anaesthetic process – considerations for the field

IS ANAESTHESIA NECESSARY?

Anaesthesia can be physiologically stressful on any animal, and a decision to sedate or anaesthetise an animal must not be taken lightly. However, with the animal immobile, risk of disease transmission (from bites and scratches) is also reduced, and the quality of samples taken will be enhanced. Ethical and welfare considerations must also be taken into account. Consider:

- Could the process you are investigating be conducted with the animal unanaesthetised?
- Is the animal trainable to provide what is required in a non stressful way, such as presenting an arm for blood sampling?
- If manual restraint is to be considered, will the stress induced in the animal be more damaging than an anaesthetic? Have health and safety issues been considered for staff? Is there ease of access to the animal for sample collection? (a

big problem if it is struggling inside a net). Is the process repeatable in the future without sedation?
- If anaesthesia is considered the best process, what regime will be employed? Will there be an effect of any anaesthetic regime on samples/ experimental parameters?

MANUAL RESTRAINT

As a general rule, Non Human Primates (NHP's) are sensitive to stress, and, except for younger animals or smaller species, are very strong and able to inflict severe injuries on human handlers. Handling is a compromise between human and animal safety in a situation where stress levels must be kept as low as possible. Physical handling without tranquillisation is possible for smaller individuals, but chemical tranquillisation is necessary for larger individuals, or for major procedures.

NHPs weighing less than 3 kg can easily be handled and manipulated by one person. However, most of these species are agile and will not hesitate to bite if they feel threatened. Always handle primates with protective leather gloves. **Rapid capture is essential**. Chasing an animal around an enclosure is psychologically stressful to the animal. Physiological stress can also be induced, which can lead to hyperthermia, which can rapidly lead to death.

The smallest animals can be gripped around the neck with the thumb and forefinger, or by a fold of skin on the flank (especially prosimians). Larger prosimians can be held by the scruff of the neck with one hand, and by the feet or base of the tail with the other (FIGURE 1). Note however that most prosimians have an explosive flight reaction when disturbed in their nest or their captive box and considerable care should be taken when capturing individuals manually (Bearder & Pitts, 1987).

Figure 1. Manual restraint of a Ring Tail Lemur. Handler Chris Yarwood (Source Chester Zoo)

Small monkeys should be grasped just under the arms, with the fingers encircling the upper chest. This position is convenient for intramuscular or intraperitoneal injection. However, for more effective immobilisation, the arms are held together behind the animals back in the same fashion as that used with larger primates (figure

2). A second person is always required to carry out procedures, and restraint should be kept to as short a time as possible to minimise stress. **Prolonged handling of small primates is extremely stressful, and we recommend considering the anaesthetic options below if the animal is going to be in hand for longer than a couple of minutes.**

Animals weighing 3--10 kg can be caught in a heavy net (or blanket or piece of fabric) and held by pinning the arms back. The two arms are held together with one hand behind the primate's back whilst the two feet are held together with the other hand (see figure 2). Never hold the animal by only one arm, as this could result in fracture or dislocation of the humerus. For obvious safety reasons, species weighing more than 10 kg need to be chemically restrained.

Figure 2. Correct monkey hold (Source, Steve Unwin, Taken at Limbe Wildlife Centre, Cameroon. Handler Jonathan Kang)

PRE-ANAESTHETIC ASSESSMENT AND PREPARATION

Preparation is essential so the animal can be immobilised efficiently and safely for the shortest period of time. EVERY MEMBER OF THE TEAM SHOULD BE BRIEFED BEFORE THE PROCEDURE AND JOBS ASSIGNED SO EVERYONE KNOWS WHAT TO EXPECT AND WHAT THEY SHOULD BE DOING DURING A PROCEDURE, BOTH IN GENERAL, AND IF PROBLEMS OCCUR.

Although modern drugs have a wide safety margin in primates, none are absolutely safe and the reaction of any individual primates to anaesthesia can be unpredictable. Therefore there is no such thing as "risk-free" anaesthesia and it follows that anaesthesia should never be undertaken lightly. Most drugs and drug combinations have advantages and disadvantages and it is important to use a technique with which the anaesthetist is familiar.

Many of the problems that can occur during anaesthesia are avoidable by careful pre-anaesthetic assessment of the patient and consideration of the conditions under which anaesthesia is to be performed. In emergencies such as the recovery of an escaped primate this is not always possible, but even in this situation thought should have been given to what will be needed in advance of the event.

The following conditions increase the anaesthetic risks:

- **old age**
- **small size**
- **obesity**
- **illness**
- **emergency situation.**

Prior to anaesthesia a primate's general condition should be assessed, its medical history considered and its weight determined, (often by estimation or from previous notes), in order to select the most appropriate drug and dose. It is also necessary to consider what type of anaesthesia is required - profound for surgical intervention, light for blood sampling or examination etc.

Consideration should be given to the possibility of dehydration, shock or the presence of disease, which may complicate the anaesthetic or require immediate attention once the primate is anaesthetised.

The physical assessment of a primate prior to anaesthesia can be difficult and may even require the use of binoculars. If any doubt exists, time spent quietly observing a primate prior to anaesthesia will be profitable. Poor water intake, diarrhoea, vomiting, sunken eyes or unusually frequent urination may suggest dehydration. Where there is a possibility of dehydration, equipment and materials should be prepared to allow aggressive fluid therapy during anaesthesia as primates rarely tolerate intravenous fluid administration when conscious.

Coughing or breathlessness may indicate a respiratory problem, and it is important to appreciate that respiratory disease is common in primates. This is especially true of the apes (and certain monkey species) which possess laryngeal air sacs. These air sacs are vulnerable to infection, and can complicate general anaesthesia if they are infected.

Weight is sometimes difficult to estimate and weighing the animal while anaesthetised is always useful.

All anaesthetic procedures should be recorded on anaesthetic data sheets. Included should be details on the initial effect and down time and the recovery times as well. The reason for the anaesthesia should be written and any treatments given. Any recommendations for changes to the anaesthetic regime should be noted. An example from Chester Zoo is presented below:

- Anaesthetic Record - Chester Zoo -

Health Status:
1. [ ] Normal
2. [ ] Abnormal

Fasting Time:
1. [ ] < 8 hours
2. [ ] 8 - 24 hours
3. [ ] 24 - 48 hours
4. [ ] > 48 hours

Activity:
1. [ ] calm
2. [ ] active
3. [ ] excited

Demeanor:
1. [ ] depressed
2. [ ] alert
3. [ ] aggressive
4. [ ] apprehensive

Weight: _____ 1. [ ] Kg
1. [ ] actual 2. [ ] lb
2. [ ] estimate 3. [ ] gm

Environ. Temp: _____1. [ ] C
2. [ ] F

Pressure/Dist : _____

Physical Status:
1. [ ] Class I normal health
2. [ ] Class II minor health problem
3. [ ] Class III major health problem
4. [ ] Class IV serious or chronic prob.
5. [ ] Class V may not survive, with or without intervention

Immobilizing Conditions:
1. [ ] Free ranging
2. [ ] Large enclosure
3. [ ] Small enclosure
4. [ ] Squeeze cage
5. [ ] Manual restraint

(a) Drug administration
1. [ ] isolated
2. [ ] in group

Body Condition:
1. [ ] obese / fat
2. [ ] good
3. [ ] fair / thin
4. [ ] poor / emaciated

(b) Location
In enclosure [ ]: inside [ ] outside[ ]
Vetlab [ ] Leahurst [ ]
Other[}_____

Accession: _____Date: ____/____/09
Species: _____
Sex: _____ Tattoo/Tag: _____
House name: _____ Birthdate/age: _____
Transponder: _____
Procedure: _____

Dose: **P**remed, **I**mmobilizing, **S**upplemental, **M**aintenance, **A**ntagonist, **O**ther
Route: **P**olesyringe, **B**lowdart, **M**etal Dart, **H**and syringe, **N**on-metal Dart, **F**acemask, **C**hamber, **E**ndotracheal **T**ube, **V**enous catheter, **O**ral, **M=**intramuscular, **S=**subcutaneous, **V**=intravenous, **P**=intraperitoneal
Del: **C**omplete, **P**artial, **N**one, % ____
Effect: **0** = no effect /fully recovered
1 = mild sedation, **2** = heavy sedation, **3** = light anaesthesia, **4** = surgical anaesthesia, **5** = excessively deep, **6** = death

Dose Class	Drug Name (generic)	Drug Strength mg/ml	Amount ml	Amount mg or %	Route	Time Given	Delivery Success	Max. Effect	Time Max. Effect	Batch / Trade Name
					/					
					/					
					/					
					/					
					/					
					/					
					/					

PHYSIOLOGICAL DATA: Temp [ ] C. [ ] F. **/** Heart (bpm) **/** Resp (bpm)
METHOD USED FOR MONITORING – VISUAL [ ] PULSE OX [ ] STETHOSCOPE[ ]

(c)	Temp	Heart	Resp	Isofl %	02 sat%	Sys/Dia/Mean	(d)	Temp	Heart	Resp	Isofl %	O2 Sat%	Sys/Dia/Mean

Initial Effect Time: _____:_____

Recumbency Time: _____:_____

Endotracheal Tube: _____

Complications:

Anaesthetic Ratings:

	Excellent	Good	Fair	Poor
Induction	1.[ ]	2. [ ]	3. [ ]	4. [ ]
Muscle Relaxation	1.[ ]	2. [ ]	3. [ ]	4. [ ]
Overall	1.[ ]	2. [ ]	3. [ ]	4. [ ]

Vet: 1. [ ] SS 2. [ ] SU 3. [ ] JAC

Recovery:
1. [ ] Normal
2. [ ] Abnormal
3. [ ] Prolonged
4. [ ] Stormy
5. [ ] Renarcotized

Recovery Data:
(initial) Time: _____:_____ Effect: ____

1. [ ] None 3. [ ] Major (fully) Time: _____:_____ Effect: ____
2. [ ] Minor 4. [ ] Fatal **Recorded by**: _____

Blood Sample Data:
Time: _____ : _____

Collection Site: L / R _____

By: 1.[ ] SS 2.[ ] SU 3.[ ]JAC 4. [ ] KH 5. [ ] TC

Haematology sample:
1. [ ] EDTA
2. [ ] EDTA – liquid
3. [ ] EDTA – dry
4. [ ] Heparin
5. [ ] Heparin – liquid
6. [ ] Heparin – dry

Chemistry sample:
1. [ ] serum
2. [ ] plasma

Comments:

_____

_____

_____

_____

_____

_____

Oxygen sat. %																																				Oxy. Sat %
Temp C/F																																					Temp. C / F
ISO %																																				ISO %
O2 (L)																																				O2 (L)
TIME	:00	:05	:10	:15	:20	:25	:30	:35	:40	:45	:50	:55	:00	:05	:10	:15	:20	:25	:30	:35	:40	:45	:50	:55	:00	:05	:10	:15	:20	:25	:30	:35	:40	:45	:50	:55	:00	:05	:10	:15	TIME
NOTES																																									NOTES

Ancillary anaesthetic monitoring equipment must be on hand. In field situations, the absolute minimum will include a competent anaesthetist, pulse oxcimeter, stethoscope, thermometer, and if possible, a capnograph.

After the procedure, all equipment should be cleaned and returned to their allocated place.

A debrief of the procedure should always take place even if the procedure has gone well to make suggestions for improvements in the future.

Preparing the Animal.

(i) **Pre anaesthetic fasting.**
Primates being anaethetised for elective procedures should be fasted to reduce the risk of vomiting and subsequent inhalation of vomitus. Except for small species, this should be for at least 12 hours (remove water 6 hours before hand). In smaller species such as guenons, 6 hours fasting is usually sufficient (2 hours for water). Care is needed in stressful situations and fasting might sometimes result in pica or coprophagy.

(ii)**Analgesia**
Analgesia should be considered for all procedures that would be considered painful in humans. Not only is this ethically sound, but the animal will recover a lot faster from an anaesthetic, with pain relief as part of the anaesthetic plan, after a painful procedure.

(iii) **Anaesthetic Doses**
Drug doses and regimes should be researched and/or previous anaesthetics on the same animal or species consulted from previous records.

(iv) **Emergency drugs and protocol**.
At every facility an emergency protocol must be in place as to what action needs to be taken if an anaesthetic does not go as planned. The standard **A**irway **B**reathing **C**irculation **D**rug therapy protocol can be employed or a variant of it. Emergency drugs such as adrenaline and atropine should be readily available, either with doses already drawn up into labelled syringes for that animal, or with dose charts readily to hand. Each facility will need to adapt any emergency anaesthetic complication protocol to their own situation. An example of this protocol, including emergency drug dosages, is presented in the emergency medicine section 3.8 and in a cut down version at the end of this section.

(v) **Animal restraint while under anaesthetic**. Some method of physical restraint may be mandatory, especially in the larger monkey and ape species.

(vi) **Airway patency during anaesthesia**
Small monkeys should be masked with Oxygen (and suitable endotracheal tubes available). Larger monkey species, and the apes, should be intubated (Figure 3 and 4).

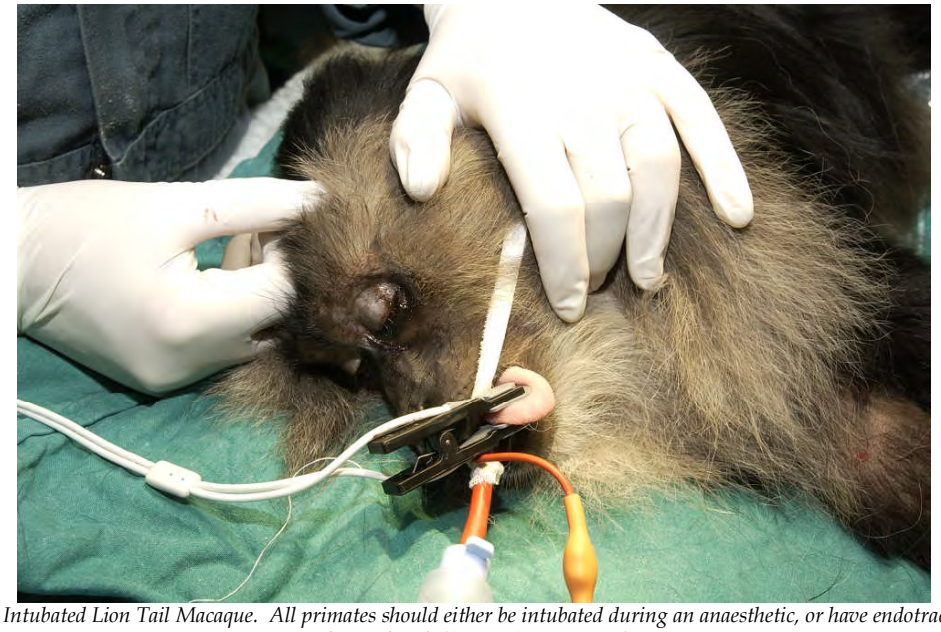


Figure 3. Intubated Lion Tail Macaque. All primates should either be intubated during an anaesthetic, or have endotracheal tubes close at hand. (Source Steve Unwin)

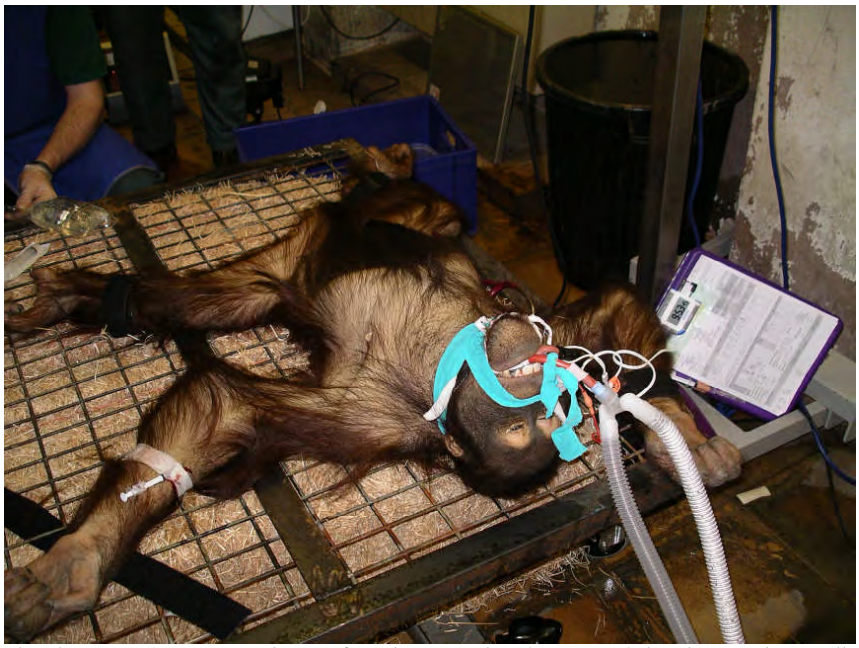


Figure 4. Intubated Bornean Orangutan. This was for a short procedure (30 minutes), but the animal was still intubated and provided with oxygen, and IV access had been obtained (Source: Steve Unwin)

ROUTE AND METHOD OF ANAESTHETIC INDUCTION AND MAINTENANCE.

This will be based on the animal's species, size, age, and state of health.
No anaesthetic works immediately and consideration must be given to what may happen during the time between administering an anaesthetic and induction of anaesthesia. If the primate is contained in a carrying box or crush cage during this period the potential for problems is minimised. However, if anaesthesia has to be induced by darting in outside enclosures the weather should be considered, pools drained, the risk of falling from platforms, trees etc after induction taken into account and unnecessary personnel kept away. ALWAYS attempt to isolate the

animal to anaesthetise it before anaesthetic induction is attempted WHEREVER POSSIBLE.

It is essential that primates are not excited prior to or during anaesthetic induction. An excited primate will require higher anaesthetic doses and take longer to become recumbent. Both these factors, plus the higher adrenaline levels circulating in excited primates increase the potential for complications during anaesthesia. Hearing is usually the last sense to be abolished by an anaesthetic, therefore it is also important to have quiet environment to avoid disturbance during induction.

In the case of primates that can be safely hand-held, anaesthetic agents can be delivered by direct intramuscular injection into the quadriceps, hamstrings or shoulder muscles. Crush cages also allow relatively safe intramuscular injection of anaesthetic agents. Oral use of medetomidine might allow hand injection. Remember that primates and especially apes recognize the person who anaesthetises them. In captivity operant conditioning can train some individuals to present a muscle mass for hand injection.

After an anaesthetic agent has been delivered, it is important to allow sufficient time for its full effect to be realised. Therefore a minimum of 10-15 minutes should be allowed to elapse before a primate is disturbed, irrespective of whether it is down, unless an emergency situation such as respiratory arrest develops during this period. Failure to allow such a period may result in partial arousal during the induction period leading to a lighter plane of anaesthesia than would normally be expected with a given anaesthetic dose rate, especially with a medetomidine/ ketamine mix.

Specific anaesthetics or anaesthetic combinations with appropriate dose rates are given in part 3.

(i) Induction using an Injectable agent:
Most anaesthetic induction agents are given via intramuscular or intravenous injection. Care is needed to ensure that primates do not injure themselves following the administration of injectable anaesthetics or during the recovery phase (see below). If an animal is remotely injected with anaesthetic (such as with a blowpipe or dart gun), they should be on the ground when darted so they are not injured if they fall. Preferably, all monkeys should be induced in crush cages. This process is quick and accurate. Enclosure design should accommodate the use of various sized crush cages. When animals are released after dosing with an injectable anaesthetic for example, they invariably sit high up on a perch or cling high up on the sides of the cage or pen, and in a large cage may fall heavily when they lose consciousness. This can be prevented by using a small cage for anaesthetic induction, or by supporting the animal as it succumbs. Note however that this last may actually increase the animals stress, and make anaesthetic induction less smooth. The decision must be based on an individual assessment.

(ii) Induction using Inhalation agent:
In small species such as guenons, and in juvenile larger monkeys, either manual restraint or an induction box can be employed and the animals masked down with inhalation agent. Handling can be very stressful to the individual concerned, particularly with an agent such as isoflurane which has an unpleasant odour and is a respiratory irritant. Sevoflurane does not have these negative aspects and should be considered in animals too sick for an injectable agent or those more amenable to handling. Little is yet published on the use of this anaesthetic in non human primates. Use of an induction box can often be a better alternative to manual restraint. For example you can create an induction box by placing a small holding cage or carrier inside an airtight plastic bag, and provide a hole to pump in gaseous anaesthetic and oxygen.

(iii) Anaesthetic Maintenance:
When it is necessary to prolong anaesthesia beyond the time allowed by a single dose of the injectable induction agent, maintenance with gaseous anaesthetic agents such as isoflurane or sevoflurane administered with oxygen via an endotracheal tube is the best option.

Great apes are best intubated in dorsal recumbency (figure 4) with the head extended off the edge of the table.

However, certain injectable anaesthetic drugs (e.g.: ketamine) can be given in incremental intravenous doses. If supplementary anaesthetics are given in intramuscular increments the total recovery time may be significantly prolonged. The IV route allows lower quantities of drugs, but as the elimination is faster, it might be necessary to top up frequently. It is therefore not recommended to EXTEND an anaesthetic by injectables alone for longer than 60 to 80 minutes.

Whatever the method of induction, placement of an endotracheal tube to maintain a patent airway is recommended in nearly all general anaesthetic cases, even if inhalation anaesthetics are not to be administered. As intubation does not present any particular problems in primates, a well fitting face mask can alternatively be employed by experienced anaesthetists. Endotracheal intubation allows precise delivery of inhaled anaesthetic agents. Intubation in small non-human primates (less than 1 kg), is straightforward, using commercially available equipment and careful positioning of the animal (Morris *et al*., 1997).

In all primates, tracheal length is relatively short, so endotracheal tubes should only be inserted to just past the larynx, to prevent bronchiole intubation and consequent over inflation of one or other lung.

In chimpanzees and gorillas, laryngeal masks could be used to minimise tracheal irritation.

The larynx can be sprayed with local anaesthetic such as lignocaine before intubation to reduce the risk of laryngospasm, particularly following induction with ketamine alone, as the laryngeal reflexes will still be functioning.
Very young or very sick primates can be induced using gas anaesthesia, or intravenous propafol.

ASSESSMENT AND MONITORING OF PRIMATES DURING ANAESTHESIA

The primary purpose of anaesthesia monitoring is to maximize the safety of an anaesthetic procedure, particularly by avoiding excessive anaesthetic depth. Physiological parameters of guenons, baboons and chimpanzees are presented in Table 1. These physiological reference ranges are altered by anaesthesia or restraint

	Guenon	Baboon	Chimpanzee
Body temperature	39°C	39° C	37°C
Heart rate	2-300/min	150/min	60-80/min
Respiratory rate	20-50/min	35/min	20-50/min
Tidal volume		50 ml	

TABLE 1: Physiologic parameters of Non human primates

Following apparent immobilisation it is necessary to assess the effectiveness of anaesthesia before any procedures (including moving the primates) are carried out. This is especially important with large individuals which could be dangerous if incompletely immobilised. It is also necessary to constantly assess the well-being of a primate during anaesthesia as problems are easier to correct if detected early. **Of the five senses, hearing is the last to go, and the first to return. Noise MUST be kept to a minimum throughout induction and anaesthetic maintenance.**

Responses to stimuli such as pinching between the toes, respiration rate, colour of mucous membranes (e.g. the lining of the mouth), pulse rate and quality, and muscle tone should all be assessed without delay after apparent immobilisation, and at regular intervals (every 5 minutes) thereafter. Wherever possible, this anaesthetic monitoring should be undertaken by a dedicated person.

During intubation, the mouth should be checked thoroughly and any food material, excess saliva or even pieces of dart or dart needles removed. Body temperature should be monitored and hypo- or hyper- thermia corrected. Hyperthermia can result from pre-anaesthetic excitement, high ambient temperatures or direct sunlight during anaesthesia, or convulsions at any stage.

A member of the team should be holding the hand of the primate throughout the procedure. The grip reflex is one of the first to return after an anaesthetic, and can be used as another indicator of an anaesthetic getting too light.

Once a primate is immobilised, place a small amount of an ophthalmic ointment onto the surface of the eyes to prevent drying of the cornea. Do not allow the sun to shine directly into the eyes of a primate at any stage during anaesthesia - irreversible damage may be done to the retina, especially if the pupils are dilated.
Whilst monitoring anaesthesia, detailed notes or charts should be kept of all measured physiological parameters, including respiratory, heart rates, body temperature, pulse quality, muscle tone and mucous membrane colour.

- pink mucous membrane is normal
- red mucous membrane might be a sign of hyperthermia
- white or pale mucous membrane might be a sign low blood pressure, vascular problem

- blue might mucous membrane be a sign respiratory problem

Capillary refill time should be less than 2 seconds; time exceeding 2 seconds is a sign of low blood pressure. Where possible, use a pulse oxcimeter to measure blood oxygenation levels.

Respiratory rates decrease during anaesthesia and increase during recovery. Access to a capnograph is useful to assess carbon dioxide levels in the expelled air (C02 should be maintained between 3.5 and 5.5% of end tidal volume – above 5.5% is an indication that increased oxygen/ IPPV is required). This in conjunction with a pulse oxcimeter will provide an excellent early warning system of pending anaesthetic issues.

With keeping detailed notes of physiological parameters, changes in the vital signs - even subtle ones - can be easily appreciated and reference may be made to a particular primate's response to anaesthesia at a later date (see an example of an anaesthetic form provided above).

Always record a primate's weight during anaesthesia so that accurate dose rates of anaesthetic agents can be calculated retrospectively.

The palpebral and pedal reflexes may also be used, depending on the anaesthetic used. While these can all be useful in determining if the animal is 'too light', they provide little information about an animal that is 'too deep' (Horne 2001). Hypoxia and hypothermia, common side effects with increasing depth of anaesthesia, are the two main causes of anaesthetic emergencies. Continuous monitoring, with pulse oxcimetry, of oxygen-haemoglobin to maintain above 85% saturation and temperature, to detect hypoxia and hypothermia, represent **a bare minimum** that must be undertaken. Anaesthetic emergencies can also occur because of hypoventilation (measure end tidal CO2 via capnography), hypotension (measure blood pressure) and cardiac arrhythmias (measure cardiac electrical activity via ECG). Careful monitoring of all these parameters allows for continuous assessment of cardiopulmonary function and provides early warning, should problems arise (Horne, 2001).

Important for long-term anaesthesia is the placement of an indwelling catheter that allows for administration of fluids and emergency drugs. Sterile technique for catheter placement is imperative (Hrapkiewicz *et al*., 1998).

A note on Body Temperature

Hypothermia may be a significant problem during surgery on primates unless care is taken to keep animals warm during anaesthetic induction and the surgical preparation time. For example, Ketamine has been shown to produce a fall in rectal temperature to 35°C within 10 minutes at an ambient temperature of 30°C. Induction in the home cage means that the animal is lying on metal mesh or bars when it loses consciousness. Any drop in temperature may then be exacerbated by clipping extensive areas of hair and by the application of large volumes of rapidly evaporating skin preparation solutions (Baskerville 1999). Intraoperatively, monkeys should have their body temperature monitored and maintained by use of a circulating heating blanket, heat pads and/or heat lamps. Warm intravenous fluids are also helpful in preventing hypothermia. Latex gloves can be filled with hot water to provide short

term hot water bottles. These however must be removed and replaced regularly, and removed totally before recovery. Hypothermia is indicated at temperatures below 36°C.
The stress of handling may also result in a rise in body temperature to, at MOST, 39.5°C. Any temperature above this, or one which doesn't reduce, usually indicates an underlying disease process.

Two commonly used anaesthetic combinations, ketamine and acepromazine or tiletamine-zolazepam (Zolitel®), were compared to identify their effects upon body temperature in cynomolgus macaques (Lopez *et al*., 2002). Thirty cynomolgus macaques previously implanted with subcutaneous telemetry devices were allocated into two groups of 15 animals. Baseline temperature data were collected for 3 days before administering anaesthesia to establish normal diurnal temperature patterns for each monkey. Each group was then anaesthetized with either ketamine-acepromazine or tiletamine-zolazepam, and their body temperatures were recorded at 15-min intervals. Both groups had marked decreases in body temperature, with the greatest decreases in the tiletamine-zolazepam group. In addition, both groups had notable post-anaesthesia elevations in body temperature that often lasted for more than 24 h post-induction.

ANAESTHETIC RECOVERY

Recovery must be conducted in as calm and quiet environment as possible. Keep the animal warm and away from drafts. Extubation of the endotracheal tube should not occur until the animal can swallow. A string can be tied to the tube so it can be removed remotely, as many primates don't provide a clue as to when they are about to awaken, and extubation is often reliant on the anaesthetists experience. Providing a favourite toy for the animal may be appropriate to help keep it calm until it is sure of its surroundings. During recovery, primates try to climb upwards as soon as they recover consciousness. Fortunately, they rapidly regain the ability to cling with their hands and feet, and support their own weight. They usually then stay quietly clinging onto something until they have fully recovered.

In hot weather, recovery areas should be cooled. Before leaving a primate to recover, the mouth and pharynx (back of throat) should be checked for saliva, food material, foreign bodies etc. The latter must be removed. Be particularly careful after dental extractions as post-extraction haemorrhage can be significant. Monitoring of pulse, respiration and temperature should continue until it is no longer safe to do so or until such interference disturbs the primate.

The primate should be left in lateral recumbency with head and neck extended and the tongue protruding from the mouth if possible. This should allow any saliva to drain from the mouth. Protect the eyes from direct sunlight. Be aware of potential hazards (water, falling from heights etc) to the primates in its immediate environment - most primates will stagger somewhat as they attempt to move away following awakening. Do not feed or provide water until recovery is complete and the primate can walk without ataxia. Recovery will take longer in fat individuals.

ANAESTEHTIC INDUCTION VIA DARTING

The most common approach to capturing wild NHPs is remote injection using blowguns or capture rifles (Figure 5). Darting can also be used to dispense medications such as antibiotics or vaccines.

Figure 5: Daninject™ rifle and darts - suitable for sanctuary use in larger monkey species, chimpanzees and gorillas (Source: Chester Zoo)

Description of various darting systems, their advantages and disadvantages can be found in Sapolsky & Share (1998) and Karesh *et al.* (1998). A brief review of using air powered darts is given below. Remote delivery systems should be used only by experienced people owing to the high risks of injury to the animal from a badly located dart, and hazards related to the pre-anaesthetic stage (e.g. falling from a tree or into water, aggression from a conspecific or a predator). NHPs also often remove the dart following impact, before the full dosage has been injected, and only a mild tranquillisation stage is achieved. In this case, the animal must be monitored until completely recovered.

Ideally primates should not be darted when another is present in the cage or enclosure.

NB: NEVER DART A PRIMATE IN AN ENCLOSURE WHERE AN ELECTRIC FENCE IS ACTIVE.

When using a blowpipe care should be taken to ensure that the anaesthetic agent is delivered intramuscularly and not subcutaneously, as the latter route may prolong the induction time or may fail to produce complete immobilisation.

Particular attention should be taken with chimps who can return darts with surprising force. All primates can remove the dart very quickly so small volumes should be used. Be careful not to inadvertently dart genital swellings in females as the risk of haemorrhage is high.

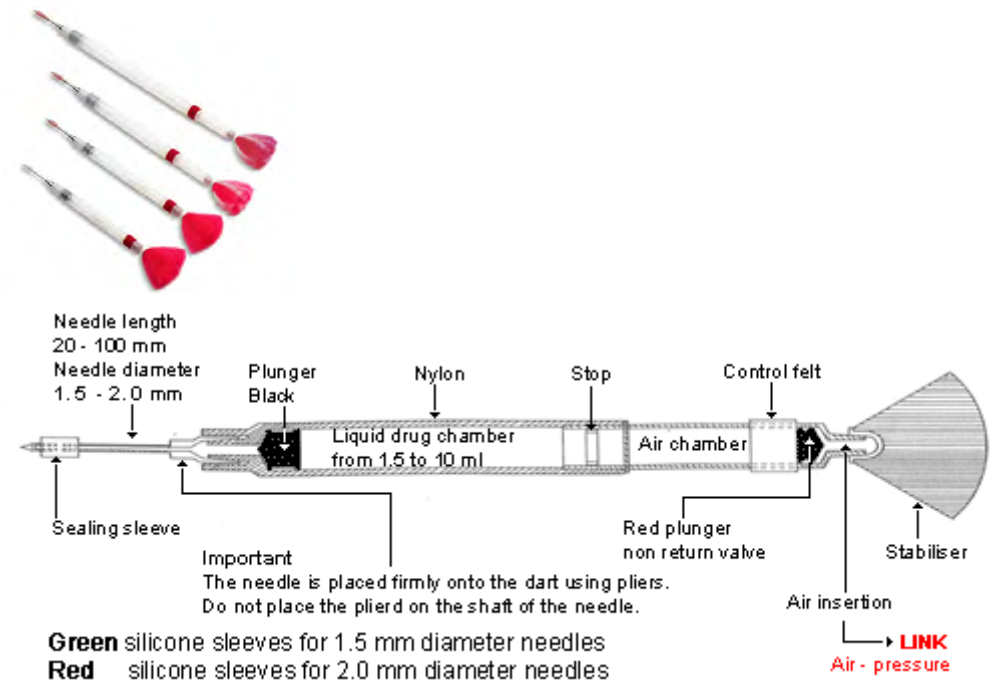


Figure 6: Components of a Dan-inject™ dart (Source: Daninject™)

Dan-inject™/ Telinject™ example - Loading the dart:

Always wear latex gloves when loading a dart. Many anaesthetics are absorbable through skin, and further precautions to prevent operator contamination may be required. CHECK THE MANUFACTUERS RECOMMENDATIONS FOR EACH DRUG.
Before attaching the needle to the dart, block the hole of the dart needle with the silicon sealing sleeve (colour depends on the size), so 1/3rd of the sleeve is above the hole(s) and 2/3rds is below it. The silicon sleeve should be used only once.
Remove the stabilizer (see figure 6) and release any air retained from the syringe air chamber.
Position the black plunger at the rear of the chamber (needle side) while holding the dart with the air chamber uppermost.
Reverse the position of the dart to put the drug chamber uppermost. Slowly inject the drug into the chamber. It is recommended you use a long needle to do this to prevent anaesthetic dribbling out of the top of the dart.
Mount the dart needle onto the dart syringe boss using pliers and locate it firmly by rotating it.

Apply a safety cap over the needle before pressurizing the dart.

Pressurising the dart:
This is best done as soon after the anaesthetic is placed in the dart as is possible. Hold the dart vertically. **Place a protective covering over the needle in case of leaks.**
Mount the coupling adapter onto a syringe. Introduce air into the syringe:
10 - 15 ml air for 1.5 ml dart
15 - 20 ml air for 3.0 ml dart
20 ml air for 5.0 ml dart
30 ml air for 10.0 ml dart

Connect the syringe to the air chamber of the dart and, with a smooth continuous action, inject the air into the dart air chamber.
The red plunger will act as a non-return valve and retain the air within the dart.
The dart is now pressurized and must be handled very carefully.

Place the red stabiliser firmly on the rear of the dart.
The stabiliser must not be wet (the syringe would be unstable in flight).
THE ONLY SAFE PLACE FOR A CHARGED DART IS INSIDE THE DART GUN OR BLOWPIPE. MOST GOOD DARTS WILL HOLD THEIR PRESSURE IN THE GUN FOR QUITE A WHILE BUT THE CHANCE OF MISFIRE INCREASES THE LONGER THE DART REMAINS PRESSURISSED. IF YOU HAVE A LOADED DART IN THE GUN FOR LONGER THAN AN HOUR BEFORE DARTING WE RECOMMEND DEPRESSURISSING AND REPEATING.

<u>After use</u>
Make sure someone (usually the anaesthetist) is tasked with retrieving all darts used as soon after anaesthetic induction as it is safe to do so. They must be depressurised immediately and placed in a safe container (e.g. a plastic tube) so they are stored safely until after the procedure.
Always clean and dry the dart and needle as soon after use as possible. Do not store drugs or water in the dart syringes for prolonged periods, it will adversely affect the plunger and dart barrel.

INHALATION ANAESTHETIC MACHINES AND THEIR MAINTENANCE

Standard to-fro systems of inhalation anaesthesia can be used in sanctuaries (Figure 7).

Figure 7– Isoflurane to-fro system with a Bain circuit attached (Source Chester Zoo).

Special inhalation anaesthetic field kits are available, (e.g. Lewis 2004, Figures 8 and 9), and provide an excellent option for the field researcher for anaesthetic maintenance.

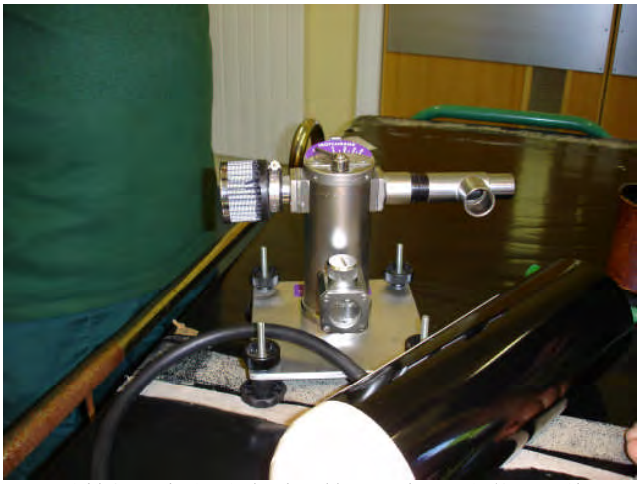


Figure 8. Field Anaesthetic Kit developed by Dr John Lewis (Source Chester Zoo)

Figure 9. John Lewis's field kit in use on an Olive Baboon at Ape Action Africa in Cameroon (Source Steve Unwin)

The maintenance of the anaesthetic machines is a critical part of successful anaesthesia.
Human anaesthetic equipment is well adapted to apes.

Regular checks should be made to make sure the anaesthetic machine is ready for use:
Check oxygen level, change if low/needle in the red.
Ensure oxygen tap is closed and any remaining pressure in the system has been released.
Remove regulator and exchange bottles.
Oxygen cylinders should be stored so they can not fall over (either lying down or chained in position), as they could explode of dropped.
Top up Isoflurane according to level gauge. AVOID breathing vapours and skin contact. Vapourisers should be recalibrated annually if at all possible.
Check for leaks: Connect circuit, close 'pop-off' valve, fill re-breathing bag and gently squeeze. Identify any leaks and rectify where possible.
Soda lime – should be replaced when changes colour or every 1-2 months depending on use. Amount of absorber should be 1.5 x the tidal volume i.e. 1000ml absorber will do 50kg animal
Circuit and re-breathing bags should be cleaned regularly (once a month), more regularly after excessive use (long procedures).

Just prior to an anaesthetic ensure all equipment is ready for use and is functional:

- Correct anaesthetic system
- Sufficient oxygen –turn on
- Sufficient anaesthetic – refill
- Masks and endotracheal tubes
- Gauze bandage or tape to hold mouth open and/or to tie tube in,
- Cuff syringe and clamp,
- K-Y Jelly,
- Lignocaine spray
- Emergency drugs are all available with dose rates and protocols.
- **Remember to regularly check for Leaks**
    - Breathing bag and or breathing circuit
    - Breathing circuit point o connection to machine
    - Breathing valves
    - CO_2 absorber gasket
    - Pressure relief valve

- Vapouriser inlet and outlet connections – filler cap
- Fresh gas delivery hose and connection to machine

ANAESTHETIC MACHINE SET-UP

See Anaesthetic machine manual and instructions.

Animals under 5kgs should use semi open system - an Ayres T piece system or Bains circuit that doesn't involve the soda-lime (figure 7).

Animals over 5kg to 100kg should use semi closed system - the circle system through the soda lime and exhaust pipe.

Semi open flow rates should be	
< 1.5kg	200ml/kg/min
Normal 30 breaths/min	200ml/kg/min
Normal >50 breaths/min	1L/min

Table 2. Flow Rates for semi open systems

Semi closed flow rates should be	IND	MAIN
25kg	1L/min	500ml/min
40kg	1.6L/min	800ml/min
75kg	3L/min	1.5L/min

Table 3. Flow rates for semi closed systems.

Body Weight	Size of Rebreathing bag
7.5kg	500mL
7.5 – 15kg	1L
15 - 30hg	2L
30 – 60kg	3L
50kg +	5L

Table 4. Rebreathing bag recommendations

A NOTE ON EUTHANASIA

Although euthanasia is never a palatable option, it is sometimes necessary for the animals' welfare. For this and staff safety reasons, primate euthanasia must ALWAYS be conducted when the animal is fully anaesthetised. A phenobarbitone overdose intravenously (femoral vein) is the most common regime. This substance is extremely irritating for the animal when injected perivascularly as it causes rapid onset tissue necrosis.

Part 2. Anaesthesia Complications and Emergencies.

Introduction - The Vital Signs of Life

A practical knowledge of a primate's **vital signs** and the physiological processes giving rise to them is essential. In simple terms, vital signs are the clinical indicators of the existence and stability of life. Every opportunity should be taken with healthy primates to observe such signs.

Life in all cells of the body depends on a good supply of oxygen and nutrients so that cellular metabolism can be fuelled. The waste products of this metabolism must be removed from the cells' immediate environment to avoid them being poisoned. Cellular metabolism, which is basically a series of highly complex chemical reactions, can only occur within a limited temperature range.

The circulatory or cardiovascular system (the heart, blood vessels and the blood flowing through them) provides the means by which oxygen and nutrients can be distributed to every cell in the body and the waste products, such as carbon dioxide (CO2) and lactic acid, removed. It is only in the extensive network of tiny capillaries that this delivery and removal service occurs, therefore normal function in the capillary network is vital for the life of any tissue.

Breathing (respiration in its narrowest sense) is the mechanism by which oxygen is taken into the lung and delivered to the red blood cells in the lungs' capillaries for distribution throughout the body. Simultaneously waste CO2 is released from the blood and voided as the primate breathes out.

A primate's vital signs provide information about the functional state of the cardiovascular and respiratory systems, thereby revealing the condition of the crucial life support mechanisms. If any component of these systems is compromised, life itself is at risk.

The vital signs of life include:

- Heart rate
- Pulse rate and strength
- Quality, rate, and gross sounds of breathing
- Mucous membranes colour
- Capillary refill time (2-3 seconds is normal)
- Core body temperature
- Volume and specific gravity of urine

Given that few serious emergencies are sudden in onset, monitoring of a patient's vital signs is the key to appropriate action during an anaesthetic**.** The same applies for a collapsed primate. Most monitoring procedures are directed towards assessing the oxygen carrying ability of the blood and the effectiveness of the circulatory system in carrying this blood to and from the tissues. Carried out correctly and with sufficient frequency, appropriate monitoring will allow you to avoid a number of deaths.

Resuscitation is not generally about dramatic rescues - prevention is always better than cure. In all anaesthetic emergency situations demanding resuscitation the actions required should follow a disciplined pattern or **ABCD** approach. Further comment will be made on this below, and also in section 3.8 (Emergency Medicine). Once faced with complete respiratory and circulatory failure (cardiopulmonary failure) restoring life is exceedingly difficult. It is good to practice these techniques so the process becomes almost automatic in an actual emergency situation.

The most significant life-threatening anaesthetic complications that are likely to be met are respiratory failure, and/or circulatory failure (including shock) and hyperthermia.

Respiratory Failure

Respiratory failure (lack of or ineffective breathing) can occur during anaesthesia or in any severely injured, shocked or diseased primates at any time, especially in cases of severe respiratory infections. During anaesthesia respiratory failure is often caused by the administration of too much anaesthetic agent, or administration of opiate drugs. It can also be caused by obstruction of the airway, or even by severe pain.

The monitoring of respiration is achieved by observing the depth and rate of breathing, by checking the colour of the mucous membranes and by ensuring that no obstructions develop within the oral cavity or pharynx. Signs of impending respiratory failure include a fall in the rate of breathing to less than 50% of normal, a progressive fall in the depth of breathing, and pallor or a blue appearance of the mucous membranes. Obstruction of the airway in unanaesthetised primates may be indicated by violent and frequent attempts by the primates to draw in breath. During profound anaesthesia, obstruction can cause inadequate ventilation without the dramatic inspiratory attempts.

As a rough "rule of thumb" complete respiratory failure in an adult primate may be considered as having occurred if there is a lack of breathing for 1 minute, with continued beating of the heart. On diagnosing this condition the following action should be taken (following an **ABCD** code):-

A is for Airway **Check that the passage of air into the lung is unobstructed**

An endotracheal tube of the correct size should be available at all times.
Remove any vomit, mucous, blood clots or foreign bodies from the mouth.
If an endotracheal tube has been fitted, check that it is not blocked or kinked.
If no endotracheal tube is in place, put the animal in dorsal recumbency extend the head and neck and pull the tongue out of the way. A laryngoscope can be useful in this situation, not only as a light source, but also to keep the tongue and epiglottis out of the way. Don't forget to inflate the cuff!

B is for Breathing **Establish and then maintain breathing**

Establish an effective pattern of breathing. This is best done forcibly using an

anaesthetic circuit and "positive pressure ventilation" with oxygen or an ambubag. However, blowing intermittently through an endotracheal tube positioned in the trachea can be extremely effective. One respiratory movement should be established every 3-5 seconds.
Intermittent pressure on the chest wall can be used to establish an airflow in and out of the lungs but is extremely inefficient for anything more than a couple of minutes. Refer to the Emergency Medicine section for more details.

C is for Circulation

Respiratory and circulatory failure often occur together and therefore it is essential to ensure that the primate's heart is still beating and that an effective pulse is present.

D is for Drugs:

The supply of gaseous anaesthetic agents should be discontinued and where reversible anaesthetic agents have been used the antidote should be administered. (The consequences of recovery to consciousness must be considered - especially where a primates is undergoing an operation).

Give atropine at 0.05mg/kg to dilate bronchioles and stabilise the heart rate.

If breathing is re-established and stabilised an anaesthetic can be continued, but monitor the primates closely and try to lighten the anaesthesia. In the case of a collapsed primates, if the respiration is restored, the cause will have to be identified and addressed. In either event close monitoring will be required for a prolonged period following the failure.

Cardiovascular or Circulatory Failure

Failure of the circulation can occur in many circumstances, but is seen particularly in severely shocked primates or those undergoing anaesthesia. It is important to realise that most anaesthetic agents have a depressant effect on cardiovascular function and anaesthetic overdoses are the commonest cause of cardiac failure during anaesthesia. The early diagnosis and treatment of cardiovascular failure is complex. For the sake of clarity and practicality only a simplified account will be given here.

1. Heart failure

"**Heart failure" means no effective output from the heart. Therefore the term includes not only complete absence of a heart beat (cardiac arrest), but also uncoordinated beating of different parts of the heart (such as ventricular fibrillation) and inefficient heart beats caused by (egg) fluid overload.**

Signs

- Absence of previously palpable pulse.
- Rapid cyanosis (blue coloration) or pallor of mucous membranes.
- No heart sound (use stethoscope).
- These signs are rapidly followed by wide dilatation of pupils, and cessation of breathing or agonal gasping.

Action

Look at watch, set stopwatch. Brain cells are particularly at risk from hypoxia and irreversible brain damage is likely unless cerebral circulation is restored within 3 minutes.

- **Airway** - check as above.
- **Breathing** - Establish and maintain breathing, Provide oxygen if possible.
- **Circulation -** Apply external cardiac massage: Intermittent pressure on chest wall over heart - 1 per second.
- **Drugs** - Turn off or reverse reversible anaesthetics.

If cardiac arrest

Adrenaline: 0.01mg/kg IV every 3-4 minutes (1ml of 1/10,000 adrenaline per 10 kgs)
Atropine: 0.05mg/kg IV

If ventricular fibrillation

Lignocaine: 1-2 mg/kg IV

If cardiac function is restored consider giving IV fluid to correct hypovolaemia and monitor very closely. Cerebral oedema often follows circulatory failure - give 1mg/kg methyl-prednisolone every 6 hours x 4, plus diuretics.

NB: The restoration of effective cardiovascular function after cardiac failure is difficult, and prevention is infinitely better than attempting a cure. A common situation during anaesthesia where too much anaesthetic has been given is the weakening of pulse strength and slowing of the heart. The timely administration of subcutaneous or very slow intravenous atropine at this point can restore both and prevent heart failure developing.

2. Shock

Shock is failure of the microcirculation (basically the capillary network) to provide adequate perfusion of the tissues with blood. Thus cells are deprived of oxygen and nutrients, and waste products are not removed. Local cellular death will eventually occur, followed by death of the animal. Various categories of shock can be recognised, but of most practical importance is hypovolaemic shock ("low blood volume shock") - due to loss of blood, plasma, or just water and electrolytes. Common causes include haemorrhage, severe and prolonged diarrhoea or vomiting, or simply the inability to drink whilst injured. Whatever the cause, the main effect is insufficient liquid in the blood vessels to keep capillaries open and functioning.

Primates suffering from lesser degrees of fluid or blood loss may not actually be in shock as various adjustments in the body's fluid distribution will have been made to compensate for these losses, hence maintaining the microcirculation. However, they are heading towards shock and such losses should be replaced. Thus the best way to deal with shock is to prevent it occurring.

Signs include:

- Weak and rapid pulse
- Pale or cyanotic mucous membranes
- Rapid heartbeat
- Hyperventilation
- Mental depression.

If the delay in treating shock is too long, damage to cells will be irreversible.

Action

- Correct any obvious cause - anaesthetic off or reversed, stop haemorrhage
- Give intravenous fluids rapidly. Give 40ml/kg of crystalloid solution such as lactated Ringers solution. Hypertonic saline (7 - 7.5% sodium chloride) can be given as an alternative. 5mls/kg**.** If dehydration is severe, <100mls/kg can be given.
- Give broad spectrum antibiotics and high dose corticosteroids (E.G: dexamethasone at <5mg/kg IV)
- Provide oxygen if available
- Monitor vital signs very closely. Especially capillary refill time and pulse rate and quality.

Always remember A,B,C,D. approach.

3. Hyperthermia

Untreated, hyperthermia can lead to brain damage and death from pulmonary oedema. Even in less extreme cases a prolonged recovery from anaesthesia can be expected.

Mild cases: Externally applied water and increased air cooling.

Severe cases: Cold water immersion, cold water enemas, IV fluids and corticosteroids plus antibiotics.

Respiratory Arrest In Brief (Print this section and have on hand during every anaesthetic)

Causes

- Anaesthetic medullary depression = too much anaesthetic agent
- CO_2 washout in hyperventilation = too much oxygen
- Respiratory muscle paralysis

Management

- Check heart and pulse rate
- Ensure airway patency and intubate ASAP.
- Ventilate with 100% oxygen (no anaesthetic agent)
  - Small mammals every 10-15 seconds
  - Large mammals every 30-45 seconds

Treatment

- Intravenous (or intramuscular) reversal agents (dependent on type of anaesthetic agent used)
- Provide supportive therapy - bicarbonate to correct acidosis

Summary

- Check presence of heart and pulse rate
- Ensure that airway is clear and intubated
- Ventilate with 100% oxygen
- Reverse the anaesthetic as much as possible

Cardiac Arrest In Brief (Means The Heart Is Not Beating). Print this section and have on hand during every anaesthetic

Causes

- Anaesthetic overdose
- Hypoxia / hypercapnaea
- Vagal reflex mechanisms causing bradycardia / asystole
- Blood loss
- Electrolyte changes, in particular potassium and calcium
- Cardiac disease

Clinical Signs

- Absence of respiration
- Absence of pulse
- Dilation of pupils
- Cyanosis of the mucous membrane and non-pigmented skin
- Lack of a capillary refill
- Colour and degree of haemorrhage from a surgical wound
- Absence of heart sounds

Treatment

- Stop anaesthesia
- Intubate and ensure patent airway: check the patient, the tube and the machine
- Administer ventilation (IPPV) immediately by bagging and check chest expands
- Begin cardiac massage.
- Ventilation : cardiac compression ratio 1 : 5 if team available 3 : 15 if sole operator
- Assess effectiveness of resuscitation by checking pupils, pulse, mucous membranes, capillary refill
- Place intravenous catheter (by direct venipuncture or by cut down with scalpel blade)
- Administer anaesthetic reversal agent
- Draw up adrenaline 1-5 ml 1:10,000 adrenaline
- Draw up bicarbonate
- Draw up short acting corticosteroids: 2-mg/kg

Summary

A - Airway	→	Intubate and ensure there is a patent airway
B - Breathing	→	Begin IPPV by bagging
C - Circulation	→	Begin cardiac massage
D - Drugs	→	Reversal agent Adrenaline

ANAESTHETIC EMERGENCY DRUG DOSES (also refer to the emergency Medicine section 3.8)	
Adrenaline	Adrenaline requires to be diluted before use because in most cases it comes packaged as 1:1,000 and we require 1:10,000. Draw up 0.1 - 0.5 ml in a 1 ml or 5 ml syringe and then fill syringe to 1 ml or 5 ml with water for injection. This will give you 1-5 ml of 1:10,000 adrenaline. 0.01mg/kg IV every 3-4 minutes (I.e. 1ml of 1/10,000 adrenaline/10 kg). IV effect is immediate, IM effect within 8 minutes
Atropine	0.05mg/kg IV
Lignocaine	1-2 mg/kg IV If ventricular fibrillation
Doxapram	20mg/ml Lge animals 0.5 mg/kg = 0.025 ml/kg. 2.5 ml / 100 kg Sm animals 5-10 mg/kg = 0.25 - 0.5 ml/kg. 2.5 ml / 10 kg
	REVERSAL AGENTS
Yohimbine	10mg/ml 0.125 – 0.25mg/kg
Atipamezole	5mg/ml 4 – 5mg per mg medetomidine 1mg per 10 mg xylazine
Naltrexone	100mg per mg carfentanil 20mg per mg etorphine

Table 5. Anaesthetic emergency drug dosages including reversal agents (also refer to section 3.8)

PART 3. Anaesthesia Agents and Suggestions by Species

Summarised anaesthetic information and dose rates are presented on tables 6, 7 and 8 at the end of this section. Suggested analgesic dose rates are presented in table 9. With all anaesthetic agents, dose rates given are only guidelines. Variations in response to these standardised dose rates will be seen in individual primates. Such variations depend on a host of factors such as general health status, pre-anaesthetic state of excitement, concurrent disease, biological variations etc.

How do you choose which Anaesthetic is best for your situation?

The choice of substance and dosage is always a compromise between safety and efficiency, and depends on the delivery system, skill and preparation of the human team, ecological conditions (e.g. temperature), and the biological features of the primate itself. The equivalent dosage of anaesthetic will have a variable effect, depending on the metabolic size of the individual. There are also wide differences in physiological characteristics (body temperature, heat depredation, heart rate, etc.) between species. Several other biological features (age, body condition, pregnancy, lactation) must also be considered.

The first priority when choosing an anaesthetic is to maximise the safety margin of the drug (the difference between an efficacious and a lethal dose), since in field conditions, the exact weight of the animal to be caught is rarely known.

a. Preanaesthetic Medications.
Analgesics, (table 8), should be given for invasive procedures that are known to cause pain in other mammals. **Buprenorphine (Temgesic)** is recommended for control of acute or chronic visceral pain but can cause sedation. **Butorphanol (Torbugesic)** is recommended for mild postoperative discomfort. Both these drugs are opiate based preparations. Note however that primates have more of an agonist effect at the Mu receptor (compared to other mammals) which may cause severe respiratory depression when using butorphanol, especially in combination with anaesthetic agents, (Ligouri *et al*., 1996).

The respiratory depressant effects of opiates and **benzodiazepines** (such as diazepam and medazolam) are synergistic in humans. Benzodiazepines alone have minimal respiratory depressant effects but, combined with even small doses of opiates, have been shown to cause respiratory collapse in some humans. Thus, these two classes of drug should not be mixed in non human primates.

Oral diazepam, used as a pre-induction agent at 1mg/kg at about one hour pre induction provides amnesia. However, clinical sedation when using this drug is highly variable between individuals, as well as species. Note that diazepam can also be given intravenously, to reduce seizuring but is not effective in primates when given intramuscularly (Horne 2001). Benzodiazepines have minimal effects on cardiopulmonary function and are generally considered very safe compounds to use in primates.

b. Injectable anaesthetics

Ketamine: Ketamine used alone as an injectable anaesthesia agent has long been a mainstay of primate anaesthetics. Ketamine would be the induction agent of choice if preanaesthetic fasting is not possible (for example if an animal has a fight wound requiring immediate attention), as gagging reflexes are maintained.

Ketamine belongs to the cyclohexamine class of drug and, is a non-competitive NMDA receptor antagonist, which is short acting and has been used as a dissociative anaesthetic as well as a research tool in psychosis (Shiigi and Casey 1999). As a dissociative agent, very high doses produce a state of catalepsy and profound analgesia. Cardiovascular function is slightly stimulated rather than depressed when ketamine is used, and there is respiratory depression only at high doses (Baskerville 1999). The pharyngeal and laryngeal reflexes are well maintained, except at very high doses, which is an advantage since primates often have to be anaesthetised when food has not been withheld beforehand (thus increasing the risk of emesis and reflux, possibly leading to aspiration). The drug may also be given on repeated occasions, though some tolerance may develop. As ketamine is frequently used for restraint for taking blood samples it should be noted that high doses decrease the leucocyte and erythrocyte values.

Animals given ketamine are often adequately anaethetised for placement of an endotracheal tube and can then be maintained by inhalation anaesthesia. If a monkey is too light to be intubated on the initial dose of ketamine, a small intravenous bolus of ketamine (at ¼ the original dose) can be given to achieve intubation for gaseous anaesthesia, or administration of oxygen alone.

Ketamine alone is not satisfactory for major surgery since at anaesthetic doses it would require potentiation to provide adequate analgesia. Muscle tone is also increased during ketamine anaesthesia, making it unsuitable for use alone for invasive surgery. It can be mixed with other anaesthetic drugs to improve the rating of anaesthetic. The most common combination is with medetomidine (table 7).

Anaesthetic Research Box – Effect of ketamine on neural conductivity and visual processing.
Ghaly *et al*. (2001) examined the effect of incrementally increasing ketamine dosages on somatosensory and neural motor volleys, recorded epidurally in response to transcranial magnetic stimulation. Their findings reflected the maintenance of a state of neural excitability under Ketamine induced anaesthesia. This helps to explain the lack of muscle relaxation and the anecdotal tolerance to this drug in a number of species.
Leopold *et al*. (2002) used optokinetic responses and functional magnetic resonance imaging (fMRI) to examine visual processing in monkeys whose conscious state was modulated by low doses (1-2 mg/kg) of ketamine. They found that, despite the animal's dissociated state and despite specific influences of ketamine on the oculomotor system, optokinetic nystagmus (OKN) could be reliably elicited with large, moving visual patterns. Responses were horizontally bidirectional for monocular stimulation, indicating that ketamine did not eliminate cortical processing of the motion stimulus. Also, results from fMRI directly demonstrated that the cortical blood oxygenation level-dependent response to visual patterns was preserved at the same ketamine doses used to elicit OKN. Finally, in the ketamine-anesthetized state, perceptually bistable motion stimuli produced patterns of spontaneously alternating OKN that normally would be tightly coupled to perceptual changes. Taken together, these results demonstrate that, after ketamine administration, cortical circuits continue to process visual patterns in a dose-dependent manner, despite the animal's behavioural dissociation. While perceptual experience is difficult to evaluate under these conditions, oculomotor patterns revealed that the brain not only registers, but also acts upon, its sensory input, employing it to drive a sensor motor loop and even responding to a sensory conflict by engaging in spontaneous perception-related state changes. This is an important finding, as ketamine alone does not provide amnesia, thus creating increased stress in the individual in future anaesthetics.

Ketamine: Medetomidine (or medetomidine equivalent) combination: This combination is also a common induction regime of non human primates. They provide an ideal injectable combination in primates for minor short term procedures (30-45 mins). Medetomidine can be reversed using atipamezole (table 7). However, anaesthetic maintenance becomes unpredictable after 30 to 40 minutes without the addition of another agent, for example, Isoflurane via intubation. When using ketamine and medetomidine, it is recommended to wait 30 minutes before the reversal administration. If the medetomidine is reversed earlier, the individual will likely wake up still under some ketamine effects and may have a turbulent and disoriented recovery.

Medetomidine is an α-2 agonist, thus produces profound sedation and analgesia by virtue of its ability to modulate neurotransmitter release in noradrenergic and serotonergic pathways of the spinal cord and brain (Horne, 2001). There is slight hypotension on administration of this drug, and this can readily be controlled by IV fluid administration. Note however that it is unsafe to use medetomidine alone, particularly in great apes, because they can readily be roused, even from a very deep sedation (see transmucosal use of medetomidine in the chimpanzee section below). Thus, a medetomidine/ ketamine combination is used (table 7).

A recent study, in a laboratory setting, compared ketamine alone with ketamine plus medetomidine for balanced anaesthesia and assessed the repeated intramuscular use of ketamine and its potential for tissue damage (Sun *et al*., 2003). The combination of ketamine and medetomidine was tested in newly arrived macaques undergoing a period of quarantine in an animal facility. Results indicated that the medetomidine and ketamine combination induced a deeper, more level plane of anaesthesia of longer duration than did ketamine alone. Furthermore, use of the medetomidine-reversing agent, atipamezole, permitted more rapid recovery.

A preliminary study in adult rats was undertaken to assess tissue damage induced by intramuscular injection of ketamine versus the combination of ketamine and medetomidine (Sun *et al* 2003). Histological evaluation of tissue inflammation and muscle necrosis in rats indicated that the lower dose of ketamine, afforded by combination with medetomidine**,** caused markedly less damage to muscle tissue at injection sites.

A major advantage with medetomidine is that its effects are reversible with atipamezole. Takako *et al*. (2001) evaluated the sedative effects of medetomidine, and a medetomidine-midazolam combination, in Japanese macaques and the antagonism of medetomidine-midazolam with atipamezole. Behavioural changes and responses to external stimuli were assessed. Animals given medetomidine became sedated but could be aroused by external stimuli. Despite the lower (25%) dose of medetomidine involved, the effects of medetomidine-midazolam were more marked. Macaques given this combination became sedated in 4 ± 2 minutes (mean ± SD) and remained unresponsive to external stimuli for at least 60 minutes. Five out of six macaques became laterally recumbent for 74 ± 37 minutes. Intramuscular atipamezole effectively reversed sedation, shortening the arousal and total recovery time. The recovery from sedation was rapid and smooth, being completed 19 ± 11 minutes after antagonism. It was concluded that the medetomidine-midazolam combination provided useful chemical restraint and may prove useful in macaques undergoing some experimental, diagnostic or therapeutic procedures.

Anaesthetic Research Box. The cardiorespiratory effects, effectiveness, and reversibility of two injectable anaesthetic combinations in captive Patas Monkeys *(Erythrocebus patas)* (Kalema-Zikusoka *et al*., 2003)**.**
Seven patas monkeys were hand-injected with medetomidine (40 micron/kg IM.), butorphanol (0.4 mg/kg IM.), and ketamine (3.0 mg/kg IM), and seven were injected with the same dosages of medetomidine and butorphanol plus midazolam (0.3 mg/kg IM). Heart rates decreased in monkeys in both treatment groups and were lower than those previously recorded in patas monkeys anaesthetized with either ketamine or ketamine and isoflurane. Mean arterial pressures were highest in ketamine-treated monkeys but remained within normal limits for both groups. End tidal CO_2 values increased gradually over time in both groups and were above physiologic norms after 20 min. Respiratory rates were similar between groups and remained constant throughout the procedures. Despite adequate ventilation parameters, initial low percent oxygen-haemoglobin saturation (SpO_2) values were suggestive of severe hypoxemia. It was not clear whether these were accurate readings or an artefact of medetomidine-induced peripheral vasoconstriction. Oxygen supplementation restored SpO_2 values to normal (>94%) in both groups. Both combinations effectively produced a state of light anaesthesia, although spontaneous recoveries occurred after 30 min in three ketamine-treated monkeys. All monkeys were given IM atipamezole (0.2 mg/kg) and naloxone (0.02 mg/kg), whereas midazolam-treated monkeys also received flumazenil (0.02 mg/kg, i.v.), which resulted in faster (median recovery time = 5 min) and more complete recoveries in this group. Both combinations were considered safe to use when supplemented with oxygen, although the midazolam combination provided a longer anaesthetic period and was more fully reversible.

Tiletamine-zolazapam (Telazol ®/ Zoletil®) is a 1:1 combination of the cyclohexamine, tiletamine, and the benzodiazepine, zolazapam. It has been widely used in primate medicine, particularly in field situations, either alone or combined with an α-2 agonist (table 7). It is a good combination for anaesthetic novices, as side effects are minimal, and the therapeutic index is wide, so potential overdosing is unlikely to cause long term problems. Tiletamine is more potent than ketamine and thus small volumes of Zoletil are required for injection. For example, in the authors experience, zoletil at 3-4mg/kg provides excellent immobilisation in chimpanzees for

60 minutes, but full recovery can take a couple of hours. Zoletil immobilised primates tend to maintain stable cardiopulmonary parameters (Horne, 2001)

Propafol (table 6 and 7), given intravenously through an indwelling catheter provides a smooth, rapid (less than 30 seconds) induction of anaesthesia and can be used to maintain anaesthesia by intermittent bolus administration – which would be required every 5-10 minutes. Primates maintained on propafol should be intubated, as propafol may induce a short period of apnoea. Monkeys recover from propafol smoothly and rapidly (Hrapkiewicz *et al*., 1998).

c. Inhalation anaesthetics

All of the common inhalation agents have been used in primates, with isoflurane currently being the most popular. Although generally acknowledged as being much safer than halothane, isoflurane does have potent vasodilatory properties that can cause severe hypotension if not used carefully (Horne, 2001). Sevoflurane (widely used in humans, but to date only rarely in non human primates) is not as soluble in the blood as isoflurane, while maintaining a 50% greater alveolar concentration, translating clinically to much faster induction and recovery times. In a calm patient, mask induction and intubation can be achieved in as little as 5 minutes (compared to 10 minutes for isoflurane). Whatever inhalation agent is used, they all cause cardiovascular depression, although, unlike halothane, this is very minimal with sevoflurane and isoflurane. All depress the respiratory system.

Anaesthetic research box. Sevoflurane use in Rhesus Macaques.
The effects of sevoflurane on cerebral metabolism and haemodynamics have been studied in rhesus monkeys (Yoshikawa *et al*., 1997). At 3.0% sevoflurane, regional cerebral blood flow (CBF) increased significantly in response to the increase in the mean arterial pressure, suggesting the inhibition of autoregulation of CBF. However, regional CBF/CMR O2 ratio was not significantly different among the cerebral regions with each condition. It could be concluded that CBF, during sevoflurane anaesthesia levels of up to 3%, might become dependent on the cerebral perfusion pressure and the changes in regional CBFs varied among the regions. On the other hand, the ratio of oxygen consumption and delivery was well maintained throughout the brain regions.

Anaesthetic Agent	Pharmacology	Pharmacokenetics	Contraindications	Adverse effects
Ketamine	GABA inhibitor. Effective to Stage II anaesthesia. No change or increased muscle tone. Increased cardiac output due to increased sympathetic tone. Retains pinnal, pedal, photic, laryngeal, corneal and pharayngeal reflexes.	Well distributed to all tissues – highest levels in brain, liver, lung and fat. Increasing the dose will increase anaesthetic duration but not the intensity	Hypersensitivity, major surgery (when used alone), hypertension, heart failure, arterial aneurysms	Respiratory depression following high doses, emesis, vocalisation, erratic and prolonged recovery, dyspnoea, spastic jerky movements, convulsions, muscular tremours, hypertonicity, opisthotonos and cardiac arrest. Eyes remain open – use Lacrilube ® or similar.
Medetomidine	Alpha adrenergic receptor α2:α1 sensitivity factor of 1620:1 (cf xylazine at 10:1). Sedation, decreased GI and endocrine secretions, peripheral and cardiac vasoconstriction, bradycardia, respiratory depression, diuresis, hypothermia, analgesia, muscle relaxation, blanched or cyanotic mucous membranes and anxiolytic effects.	Onset of effect – 5 mins post IV, 10-15 mins post IM. Can be absorbed via the oral mucosa.	Animal MUST BE CALM BEFORE INDUCTION WITH MEDETOMIDIN E. Cardiac disease, respiratory disorders, liver or kidney disease, shock, severe debilitation, stress due to heat, cold or fatigue	An extension of the pharmacological effects – bradycardia, occasional AV blocks, decreased respiration, hypothermia, urination, vomiting, hyperglycaemia, pain on injection. Rare effects have also been reported including prolonged sedation, paradoxical excitation, hypersensitivity, apnoea and death from circulatory failure.
Zolazepam/ Tiletamine	Retains reflexes as for Ketamine	Onset of action is variable, but fairly rapid – 6-10 minutes	Pancreatic disease, severe cardiac or pulmonary disease	Hypersalivation in some species (use atropine to counter at induction), transient apnoea, erratic and/or prolonged recovery.
Propafol	Short acting hypnotic – method of action is not well understood.	Onset usually less than 1 minute post IV injection. Its short duration of action (10 minutes) is due to rapid redistribution from the CNS to other tissues	Hypersensitivity. Caution in pregnant or lactating animals.	High incidence of apnoea with resultant cyanosis if propafol is given too rapidly**.** Give slowly (23% of calculated dose every 30 seconds until desired effect. SINGLE USE ONLY. Extravasation of injection is not irritating nor does it cause tissue sloughing.
Diazepam	CNS depressant (anxiolytic, sedative, skeletal muscle relaxant, anticonvulsant)	Peak levels 30 minutes to 2 hours following oral administration. Slower following IM injection	Too rapid injection of IV diazepam, in small animals or neonates, may cause cardiotoxicity secondary to the propylene glycol in the	Very variable effects

			preparation. Hypersensitivity. Caution in pregnant or lactating animals.	
Isoflurane	MAY interfere with the function of nerve cells in the brain. Induces CNS depression, depression of body temperature regulation centres, increased cerebral blood flow, respiratory depression, hypotension, vasodilation, and myocardial depression (less so than with halothane) and muscular relaxation	Rapid absorption from the alveoli to the brain. Most (99.8%) is eliminated via the lungs. The remainder is metabolised in the liver	Malignant hyperthermia. Use with caution in animals with CSF or head injury and during pregnancy (may be fetotoxic)	Hypotension (secondary to vasodilation) is considered to be dose related. Dose dependent respiratory depression, GI upset, cardiac arrhythmias.

Table 6 (After Plumb 1999). Pharmacological information for several anaesthetics used in Non Human Primates

Indication and Drugs	Dosage and Route of Administration	
Sodium Pentobarbital, C-II	20-30 mg/kg	IV
Sodium Thiopental, C-III (2.5%)	15-20 mg/kg	IV
Thiamylal Sodium, C-III (2.5%) (Surital®, Bio-Tal®)	25 mg/kg	IV
Ketamine (Ketaset®, Vetalar®)	**5-20mg/kg**	**IM**
Ketamine/Diazepam:		
Ketamine	5-25 mg/kg ; 10mg/kg	IM
Diazepam (Valium®)	0.5 mg/kg; 7.5mg/kg	IM/ IV
Ketamine/Xylazine:		
Ketamine	7 mg/kg	IM
Xylazine (Rompun®)	0.6-1.0 mg/kg	IM
Ketamine/ medetomidine:		
Ketamine	**5-7.5mg/kg**	**IM**
Medetomidine (Domitor/ Zalapine®)	**0.033-0.075mg/kg**	**IM**
Propafol	2-4 mg/kg; baboons for induction	IV to effect (slowly)
	2.5-5 mg/kg; macaques	IV to effect (slowly)
	5-10 mg/kg	IV to effect (slowly)
Tiletamine + zolazapam (Zolitel/ Telazol®)	**1-15 mg/kg (higher dose rate for smaller species)**	**IM**
	2-6 mg/kg	
Tiletamine + zolazapam/ medetomidine:		
Tiletamine + zolazapam	**1.25 mg/kg (Apes)**	**IM**
	2mg/kg (chimps)	
Medetomidine:	**0.03-0.04 mg/kg (Apes)**	**IM**
	0.02mg/kg (chimps)	
Methoxyflurane (Metofane®)	To effect	IH
Halothane (Fluothane®)	To effect	IH
Isoflurane	**To effect**	**IH**
Sevoflurane	**To effect**	**IH**
Halothane/Nitrous Oxide (50% O_2 + 50% N_20)	To effect	IH

TABLE 7: General Anaesthesia regimes used in Non human Primates - those in bold recommended by the authors (see text)

Reverse Medetomidine with Atipamezole at 0.1-0.25 mg/kg (equal volume to medetomidine 1mg/mL)

NB. Erythrocebus patas may require a higher dose of xylazine. Cercopithecus sp require a much lower dose of barbiturates.

Indication and Drugs	Dosage and Route of Administration	
Morphine, C-II	0.5-2.0 mg/kg q4h	SC IM IV
Oxymorphone, C-II		
Old World Primates	0.15 mg/kg	SC IM IV
New World Primates	0.075 mg/kg	SC IM IV
Flunixin meglumine	**0.3-1mg/kg q12-24h**	**SC, IV**
Meloxicam (Metacam ®)		
Meperidine, C-II (Demerol®)	2-4 mg/kg	IM
Pentazocine, C-IV (Talwin®) not to exceed total dose of 60 mg	1.5-3.0 mg/kg q3-4h	IM SC
Buprenorphine (Temgesic®)	**0.01-0.03 mg/kg q8-12h**	**IM, IV**
Acetylsalicytic Acid (Aspirin)	10-20 mg/kg q6h	PO
Acetaminophen	10 mg/kg q8h	PO
	5-10 mg/kg q6h	PO
Butorphanol tartrate (Torbugesic®)	**0.025 mg/kg q3-6h**	**IM**

***Table 8: Analgesia suggestions for Non human primates*.**

Chimpanzees

Chimpanzees should be appropriately fasted for food and water prior to scheduled procedures requiring sedation or anaesthesia. Following sedation or anaesthesia, food and water should continue to be withheld until the animal has regained full consciousness. Selection of an anaesthetic is often based on the nature of recovery of individual animals; some exhibit severe motor movements and apparent hallucinations, and should be monitored carefully. Recovery cages, that restrict movement and permit access to the animal, should be used. Animals should not be returned to their home cage until they are fully recovered. Veterinary and care staff should learn the response of each animal to various procedures and sedatives and be aware that other chimpanzees, within the compound or in an adjacent cage, might attack a sedated animal. Recognition of pain in chimpanzees, and the need for analgesics, is best achieved by care-givers who know species-typical chimpanzee behaviours, and who are sufficiently familiar with the individual to recognize subtle changes in behaviour and mood (Fritz *et al*., 1999).

Diazepam has been used by some as a pre-requisite to immobilizing chimpanzees. One regime suggested has been 5mg per animal for juveniles and 10-15 mg per animal for adults, for 5 nights prior to immobilization with ketamine. However, this regime produces a 'calmer' animal, rather than drowsiness. An alternative to pre-induction sedation in chimpanzees is training them to accept hand injections.

Induction recommendations: Ketamine: medetomidine combination or Zoletil or Zoletil: Medetomidine combination.

For major surgical procedures in chimpanzees, properly administered inhalant anaesthetics, such as isoflurane or sevoflurane, are recommended, and offer many advantages over other anaesthetics, especially as they provide ready access to the respiratory system if the animal stops breathing. IV catheterisation should also be considered as standard procedure for all surgeries. Six adult female chimpanzees (*Pan troglodytes*) were anaesthetised for the placement of intrauterine contraceptive devices, microchips for identification, routine blood sampling, and physical measurements (Adams *et al*., 2003). Anaesthesia was induced with medetomidine, in combination with ketamine, administered by intramuscular injection with a projectile syringe. Induction was smooth and rapid but five of the animals were insufficiently relaxed for endotracheal intubation. The plane of anaesthesia was deepened by administering isoflurane delivered in oxygen and nitrous oxide, and general anaesthesia was maintained for up to 74 minutes. The action of medetomidine was reversed with atipamezole, at the end of each procedure, and the animals recovered smoothly and uneventfully.

Chimpanzees can develop tolerance to ketamine, especially if they must be frequently immobilised. In addition, there are times when the injection appears to have no effect and it must be considered that the dose had been injected into fat or muscle fascia: in these cases there might also be a prolonged recovery period.

Telazol/ Zolitel, a 1:1 mixture of cyclohexamine tiletamine (2-3 times more potent than ketamine), and the benzodiazepine zolazapam, are used for diagnostic procedures and short anaesthesia, when deeper anaesthesia and fewer muscular

contractions are needed than those achieved with ketamine alone. Note however that recovery time is extended beyond that of ketamine.

Anaesthetic Research Box. Transmucosal sedation in chimpanzees.
A new sedation regime for juvenile chimpanzees has been developed by Carmen Vidal of HELP Congo, in her work with orphan chimpanzees. Medetomidine (Domitor 1mg/mL, Zalopine 10mg/mL) is an agonistic α-2 adrenergic drug with tranquilising, myorelaxant and analgesic properties. Carmen has been using it transmucosally since 1998, and has now had her work published (English version TBP). She has found that a dose of 75ug/kg PO (cf < 50ug/kg IM/V) works well. This was confirmed by the author in a series of anaesthetic workshops in 2003. A dose range, depending on the individual animal, is given as 50-100 ug/kg. N=60.
Clinical signs include bradycardia, hypovolaemia, temperature reduction to 35C, while side effects include vomiting (on 2 occasions) and muscle tremors (in 10 of the animals).
The dose MUST be aliquoted at 1 drop every 30 seconds onto the oral mucosa just inside the lower lip (thus some training is required). It must not be put in the food, as this is ineffective. The drug can be mixed with a small amount of chocolate or jam – something the animal will keep in the mouth.
Effect is seen after 30-45 minutes (15-20 minutes if the animal is very quiet). All reflexes are still present – useful if the animal climbs as it is less likely to fall, but laryngeal reflex also remains making intubation, if required, tricky. General procedures can be performed for up to 1 hour. The animal will stay quiet for up to 3 hours if no reversal is given.
The antagonist, atipamezole (Antisedan) is given at 150ug/kg IM/IV (about 2/3-3/4 the domitor volume). Reversal is rapid and complete. Reversal must be delayed at least 20 minutes after final domitor drop, to avoid secondary medetomidine effects due to delayed oral adsorption.
Top-ups – anaesthesia can be prolonged with 1/3rd of the original medetomidine dose IM, with the addition of ketamine at 5mg/kg IM.

Gorillas

Gorillas can be anaesthetised with the same anaesthetic drugs, at similar dose rates to chimpanzees. In the authors experience, Zoletil (Telazol) at a dose rate of 1mg/kg (so half that published) has been enough for light anaesthesia and short procedures. All gorillas should be intubated.

If inhalation anaesthesia is to be used in large specimens (over 170kg), large animal circuits, such as those used for foals, or custom anaesthetic kits (see figure 10) may well be needed. However, animals up to 170kg can be maintained for procedures of up to 90 minutes on the gaseous anaesthetic field kit (figure 8).

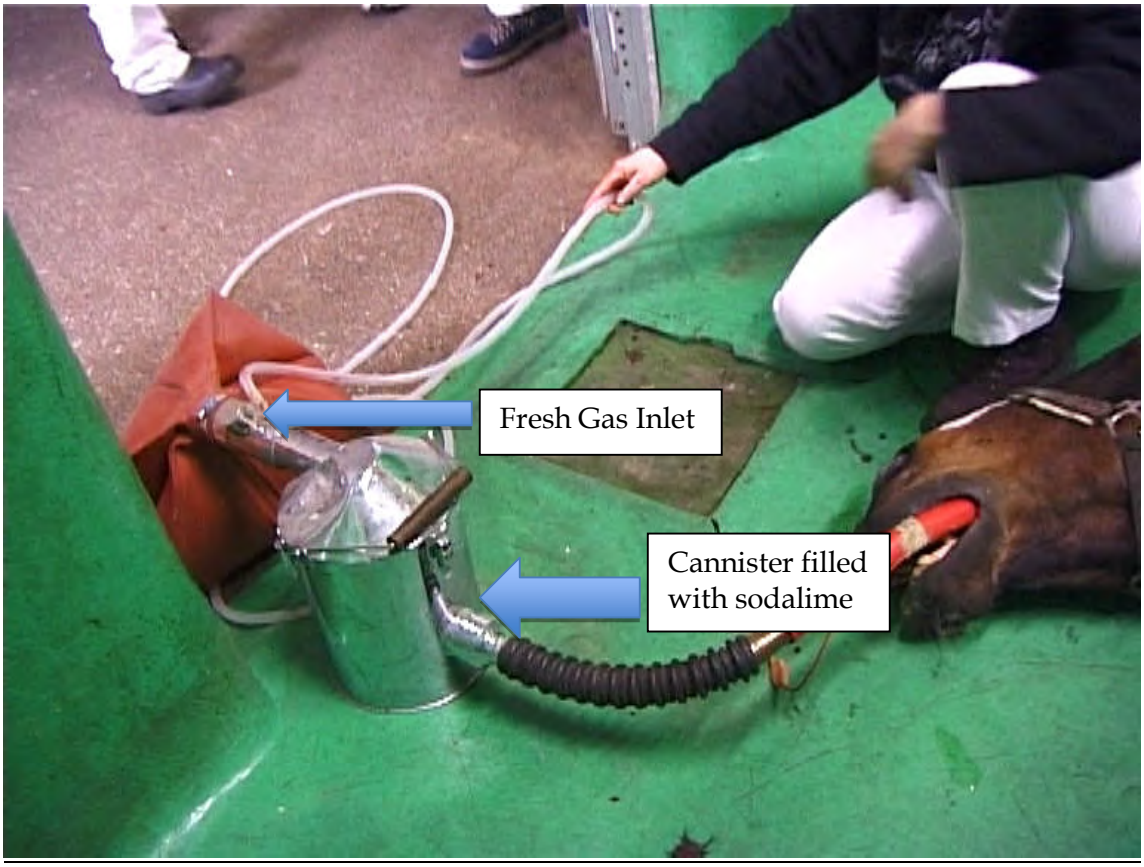


Figure 10. Custom anaesthetic kit used at Vienna Veterinary School for animals 150kg and above (Source Martina Mosing)

Table 9. CHIMPANZEE AND GORILLA ANAESTHESIA AND SEDATION and ANAESTHEIC REVERSAL.

Drugs	Dose Rate	Comments
Diazepam	1.0-1.5mg/kg ORAL	Sedation only. Pre-medicant approx 1 hour before anaesthesia with medetomidine and ketamine. Variable effect but can be used to reduce anxiety for hand injection, produces retrograde amnesia (i.e. cannot remember the events of anaesthesia) **and** it reduces the dose of medetomidine and ketamine (see below) by half. Prolongs the effect of the anaesthetic drugs and has hangover effect. Post recovery vomiting seen in Chimpanzees.
Droperidol	2.5-5.0 mg per adult 1.3-2.5 mg per juv	Sedation only. Takes 45 mins to 60 mins to take effect. Gastric absorption only.
Ketamine	7-10mg/kg IM	Short acting. Can be given as a top up of other anaesthetics at 2-4 mg/kg Induction 3-10 minutes, recovery within 40 to 60 minutes Can have sedative effect when given orally
Ketamine + Midazolam	7-10mg/kg IM + 0.05 mg/kg IM	Excellent muscle relaxation. Prolonged recovery.
Ketamine + Xylazine	10mg/kg IM + 1mg/kg IM	Good relaxation. Can be reversed with atipamezole IM or at 0.3 to 0.4 total dose of xylazine.
Medetomidine	0.075 – 0.1mg/kg ORAL	Oral sedation (transmucosal absorption) – effectiveness is increased if not mixed with anything and if the animal is on an empty stomach.
Medetomidine+ Zoletil	0.02mg/kg IM + 2mg/kg IM+	Excellent muscle relaxation. Medetomidine can be reversed with atipamezole at a dose rate of 3-5 times the total dose of medetomidine IM. Can be given 50% IV and 50% IM.
Medetomidine+ Ketamine	0.03-0.05mg/kg IM + 3-4 mg/kg IM	Good muscle relaxation. Reversed with atipamezole at a dose rate of 3-5 times the total dose of medetomidine IM. Can be given 50% IV and 50% IM.
Zoletil	3-5mg/kg IM (1mg/kg in gorilla) 15mg/kg PO	Anaesthesia is dose dependent. Agent of choice for escaped or aggressive chimps. Can be made up to 500mg/ml Rapid induction 1-7 min, prolonged recovery 1-5 hours with drowsiness, dizziness and ataxia. Lower blood pressure. Oral sedation – variable effect
		REVERSAL DRUGS
Atipamezole - Reversal for medetomidine and xylazine	0,1-0,5 mg/kg IM or IV 4x medetomidine injected	Decrease the doses regarding time elapsed after medetomidine administration
Flumazenil - Reversal for benzodiazepine	0,3mg/kg slow IV, then 0,1 to 0,2mg every minute	Increase alertness but don't significantly enhance the speed or quality of recovery.
Naloxone - Reversal for opiates (can also use Naltrexone)	1 to 2 µg/kg IV repeat after few minutes regarding effect	Can treat respiratory distress due to opiate use, but also has own set of adverse effects
yohimbine	0,125-0,25 mg/kg IM	Reversal for xylazine

Mandrills, Drills, Mangabeys, Guenons and Baboons

Induction recommendation: Ketamine: medetomidine combination or Zoletil or Zoletil: Medetomidine combination. Small species, isoflurane or sevoflurane induction in a chamber.
Injectable anaesthetics should be used for induction and anaesthesia can be maintained at 1-1.5% isoflurane or sevoflurane in oxygen.
Ketamine is the most commonly used agent, both for chemical restraint and for induction of anaesthesia in all but very young animals. It can be given intramuscularly, in small volumes, and has a wide safety margin. The ability to bite is lost at low doses and at an early stage of induction. Doses of 5-10mg/kg produce effective sedation for handling and close examination and for minor procedures, such as taking blood samples or passing a nasogastric tube. Doses of 10-25mg/kg give sufficient depth of anaesthesia for minor surgical procedures.
Due to the increased muscle tone when using ketamine alone, analgesia with good muscle relaxation can be produced by combining ketamine (5mg/kg) with medetomidine (50ug/kg), an alpha 2 agonist – see more specific dose rates below. This combination also has a wide therapeutic index, and is useful in any of the larger monkey species.

When using ketamine and medetomidine, it might be better to wait 30 minutes before the reversal administration: if done earlier, the individual will wake up under ketamine effect and might have a turbulent and disoriented behaviour.

What follows are some injectable anaesthetic doses for monkey species

ZOLETIL (Tiletamine + Zolazepam)

Mona guenons (*Cercopithecus mona*)
4-5mg/kg recommended for immobilisation. Anaesthesia can be prolonged with Ketamine or isoflurane gas. I have used doses in the range of 3.0 - 4.8mg/kg without serious complications. Doses of 3.0 - 3.9mg/kg may give less satisfactory results. Be careful and give attention to details when anaesthetising fat monas.

Red-eared guenon (*Cercopithecus erythrotis*)
3.6mg/kg provided about 40minutes of anaesthesia/immobilisation.

Putty-nose guenon (*Cercopithecus nictitans*)
We have not used Zoletil in this species. Kreeger in 'Handbook of Wildlife Chemical Immobilisation' recommends 4.4mg/kg and supplementation with Ketamine at 4.4mg/kg.

Red-capped Mangabey (*Cercocebus torquatus*)
3mg/kg will give immobilisation of short duration but this is not suitable for any painful procedure. 5mg/kg will produce surgical anaesthesia. Anaesthesia can be prolonged with ketamine or isoflurane. Giving additional zoletil to prolong anaesthesia will result in very prolonged recovery periods.

Medetomidine-Ketamine combination (NB for small guenons, induction using isoflurane in an induction chamber is recommended if it is available)

This drug combination should not be used for escaped animals. Excitement just before anaesthesia (though difficult to eliminate in guenons - in my experience) significantly reduces the effect of medetomidine and ketamine combinations.

Animals intended to be anaesthetised with this drug combination should be isolated at least 24hours before the anaesthetic event. However, depending on the animal, being isolated from the rest of the group can cause stress or excitement.

The major advantage this anaesthetic regime offers is that it can be reversed with atipamezole (Antisedan™). Atipamezole is generally administered at five times (5x) the dose of medetomidine given (thus an equal **volume** if using domitor™ or dexdomitor™).

Anaesthesia induced by medetomidine-ketamine combination can be prolonged by ketamine or isoflurane. Awakening from anaesthesia induced by this drug combination can be sudden and abrupt and animals are dangerous even at this point as they can inflict serious bite injuries.

Mona guenon (*Cercopithecus mona*)

Medetomidine: 72.5 - 103.3ug/kg
+
Ketamine: 3.6 - 5.2mg/kg

This combination in my experience often causes apnoea (temporary cessation of breathing), bradyarrythmia and atrioventricular block in monas. Apnoea usually resolves spontaneously while bradyarrhythmia and atrioventricular block resolves after reversal with atipamezole. The higher doses are more likely to cause frequent apnoea.

Red-eared guenon (*Cercopithecus erythrotis*)
Medetomidine: 54.3 - 113.6ug/kg
+
Ketamine: 2.7 - 5.7mg/kg
Lowest doses produced light anaesthesia of short duration not suitable for painful procedures. Can cause apnoea of short duration, atrioventricular block and arrhythmia.

Putty-nose guenon (*Cercopithecus nictitans*)
Causes occasional apnoeas which resolves on its own. A combination of medetomidine 73.2ug/kg + Ketamine 3.7mg/kg did not produce safe immobilisation in an adult female with a fractured mandible. A combination of medetomidine 78.9ug/kg + ketamine 3.9mg/kg produced very light anaesthesia in another adult

female. I will recommend a combination of medetomidine 80 - 100ug/kg and ketamine 4 - 5mg/kg for Putty nose guenons.

Preuss's guenons (*Cercopithecus preussi*)
Medetomidine: 64.5ug/kg
+
Ketamine: 3.2mg/kg

Red-capped mangabey (*Cercocebus torquatus*)
Medetomidine: 75 - 100ug/kg
+
Ketamine: 3.75 - 5mg/kg

Sclater's guenon (*Cercopithecus sclateri*)
Medetomidine: 63.6 - 100ug/kg
+
Ketamine: 3.2 - 5mg/kg

Combinations of 100ug/kg medetomidine and 5mg/kg ketamine can cause severe cardiorespiratory depression and I do not see the need to use this high dose in this species.

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MAJOR VETERINARY ANAESTHETIC MANUFACTURERS:
Pfizer (Europe, USA, East Africa): Domitor, Dexdomitor, Zalopine (As Orion – Finland). Pfizer have lost the patent rights to 1mg/mL medetomidine – check for cheaper alternatives from rivals. Antisedan
Fort Dodge (Europe, USA): Ketaset, Ketamine
Abbott (Europe): Isoflurane
Kyron Labs (Benrose, South Africa Ph 011 618 1544): 10mg/mL and 40mg/mL medetomidine, Naltrexone.

3.14 FLUID THERAPY IN PRIMATES

Steve Unwin

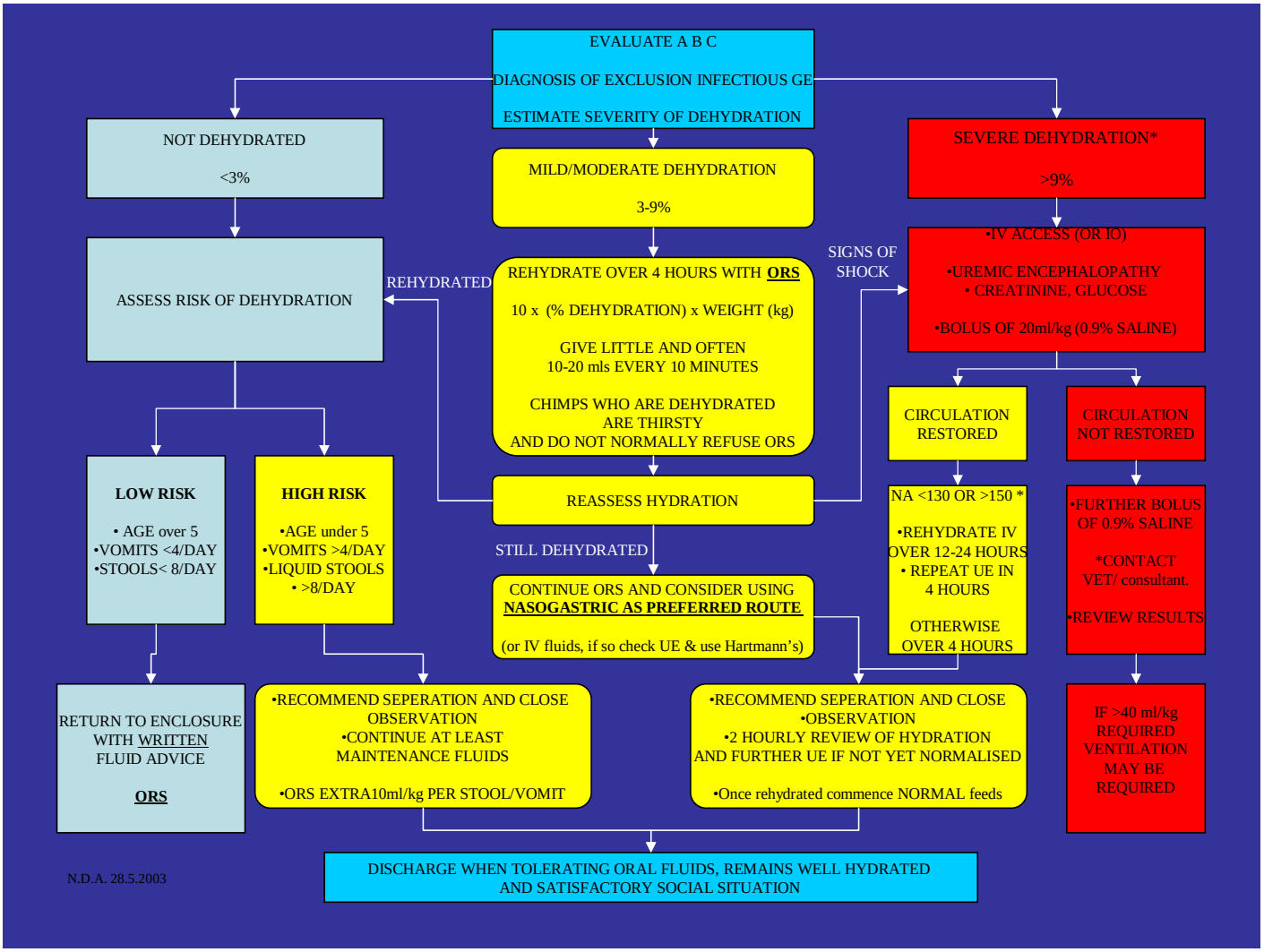


Figure. Overview of Rehydration Therapy and Principles.

INTRODUCTION

The aims of fluid therapy are rehydration, replacement of ongoing losses, and maintenance. In sanctuaries, one of the most common causes of dehydration is diarrhoea, and, after briefly discussing other needs for fluid therapy, this is concentrated on in this booklet. The booklet is divided into 3 sections. The first looks at general principles of fluid therapy, the second on oral rehydration therapy and the third on parenteral fluid therapy. Note that treatment of malnutrition, itself another major cause of dehydration, is dealt with in a separate booklet.

GENERAL PRINCIPLES

Distribution of Body Fluids

Total Body Water = 60% body weight, half is extracellular, half is intracellular. Of the extracellular, 5% body weight is intravascular (plasma) and 15-25% body weight is interstitial fluid.

Blood volume = 7-10% body weight

Parenteral vs. oral fluids

Regulation of water and electrolytes (and thus blood volume) is a function of the kidney. If there is not enough blood flow to the kidney, this regulation breaks down. However, if the kidneys are working, and the animal is not excessively vomiting, oral administration of fluids is best – the kidney is better at working out the animal's requirements than most vets! The commonest situation where oral fluids are used is diarrhoea.

In most other circumstances, intravenous administration (via a large bore indwelling catheter) is best. If the animal's blood volume is low (so low pressure), a vein may be hard to catheterise. The answer is to do a sterile cut down onto the vein; this is quicker and better for the animal than prolonged poking about the region of the vein followed by the administration of fluids by an inappropriate route.

If all else fails, fluids can be given intraperitoneally. Since the peritoneal cavity is only a potential space under normal circumstances, the chances of hitting an organ with the needle are high.

Subcutaneous administration is not a good way to give fluids because if the animal has a low blood volume/ acidosis the blood vessels supplying the skin will be constricted. This is done to divert blood to vital organs but will also mean that the fluid will not be absorbed. Some fluids (dextrose 5%) will **cause** vasoconstriction and actually draw fluid out of circulation into the subcutaneous depot! Also, apes in particular don't have a large subcuticular space.

Intraosseous administration has been used in puppies, kittens, birds, reptiles and baby monkeys. Injections via this route are **extremely painful in man**, when a neonatal animal is so sick as to be unable to fight back it is hardly sporting to subject it to excruciating pain as well. Infection is another problem when using this route – the consequences are likely to be disastrous.

TABLE 1. WHICH FLUIDS TO USE FOR WHICH SITUATION

Condition	Loss	Fluid Used
Haemorrhage	All blood components	MILD – colloids (crystalloids) SEVERE – (fresh) whole blood
Dehydration (not drinking enough)	Water	NaCl 0.18% + dextrose 4%, dextrose 5% (KCl 10-20 mmol/L added after 2 days)
Vomiting	Water, H+, Na+, K+, Cl-	NaCl 0.9%, Ringers (KCl 10-20 mmol/L added after 2 days)
Diarrhoea	Water, HCO3-, Na+, K+, Cl-	Oral fluids (see Section 2), LRS (KCl 10-20 mmol/L added after 2 days)
Severe vomiting and diarrhoea	Water, HCO3-, Na+, K+, Cl-	Colloid + LRS
Peritonitis	Plasma + ECF	Colloid + LRS
Gut Obstruction	Water, HCO3-, Na+, Cl-	Colloid + LRS + NaHCO3
Urethral obstruction	Retention of H+, K+	NaCl 0.9% + dextrose 5%

PRINCIPLES OF FLUID THERAPY

Fluid therapy is based on an assessment of the degree of dehydration present. Principles are as follows:

- *No dehydration* - If diarrhoea is present, but urinary output is normal, the normal diet may continue with fluid intake dictated by thirst. High osmolality fluids such as undiluted juices should be avoided, and maintenance oral electrolyte solution (Na 45-60 mmol/L) offered "ad libitum."
- *Mild* - If symptoms and signs are limited to decreased urinary output and increased thirst, mild dehydration is suspected. Assessment and treatment under close supervision are indicated. Rehydration consists of ORS or maintenance solution 10 mL/kg/hr with reassessment at 4-hour intervals. Early refeeding is recommended. Extra ORS or maintenance solution (e.g., 5-10 mL/kg) may be given after each stool if diarrhoea persists.
- *Moderate* - If at least two of the following signs, sunken eyes, loss of skin turgor ("tenting" of abdominal skin lasting less than 2 seconds), or dry buccal mucous membranes are present, moderate dehydration is diagnosed and rehydration consisting of ORS 15-20 mL/kg/hr with direct observation and reassessment at 4-hour intervals. If dehydration is corrected, therapy for

ongoing losses and maintenance are continued as outlined above. If not, treatment is repeated as indicated by clinical signs or symptoms.

- *Severe* - If, in addition to signs of moderate dehydration, there is rapid breathing, lethargy, coma, a rapid thready pulse or "tenting" of the skin lasting more than 2 seconds, severe dehydration and shock are present. Blood pressure should be measured. Prompt intravenous therapy is indicated with rapid infusion of saline plasma or colloid sufficient to replete blood volume (10-20 mL/kg over 30 minutes may be necessary). Intraosseous infusion should be used if an intravenous line cannot quickly be inserted.

TABLE 2. CLINICAL SIGNS OF DEHYDRATION

% DEHYDRATION	CLINICAL SIGNS
< 5	None detectable. Rely on history
5-6	Subtle loss of skin elasticity
6-8	Definite delay in return of skin to normal position +/- Eyes sunken into head (reduced fat content) +/- Dry mucous membranes
10-12	Tented skin stands in place Prolongation of CRT Eyes sunken into orbits Dry mucous membranes +/- signs of shock (increased heart rate, cool extremities, weak pulse)
12-15	Definite signs of shock – death imminent unless rapid therapy

THE PHYSIOLOGICAL PROCESS DURING DIARRHOEA.

In the normal healthy intestine, there is a continuous exchange of water through the intestinal wall – in humans up to 20 litres of water is secreted and very nearly as much is reabsorbed every 24 hours - this mechanism allows the absorption into the bloodstream of soluble metabolites from digested food.

In a state of diarrhoeal disease the balance is upset and much more water is secreted than is reabsorbed causing a net loss to the body which can be as high as several litres a day. In addition to water, sodium and other minerals are also lost (see table 1). The body's store of sodium (in the form of sodium ions Na+) is almost entirely in solution in body fluids and blood plasma, i.e., extra cellular (ECF)- by contrast 98% of the body's total potassium (K+) is held within cells i.e. intra-cellular (ICF).

The concentration of Na+ in the extracellular fluid has to be held to within close limits (135-150 mmol/l) for the proper functioning of the body. As mentioned above sodium concentration is normally precisely controlled by the renal function, however in a state of dehydration water is conserved by anuria (no urine production) and the sodium regulation cannot work effectively.

Thus continued diarrhoea causes rapid depletion of water and sodium, which is to say a state of dehydration. If more than 12% of the body's fluid is lost death occurs.

Simple giving a saline solution (water plus Na+) by mouth has no beneficial effect because the normal mechanism by which Na+ is absorbed by the healthy intestinal wall is impaired in the diarrhoeal state and if the Na+ is not absorbed neither can the water be absorbed. In fact excess Na+ in the lumen of the intestine causes increased secretion of water and the diarrhoea worsens.

If glucose (also called dextrose) is added to a saline solution a new mechanism comes into play. The glucose molecules are absorbed through the intestinal wall - unaffected by the diarrhoeal disease state - and in conjunction sodium is carried through by a co-transport coupling mechanism. This occurs in a 1:1 ratio, one molecule of glucose co-transporting one sodium ion (Na+).

It should be noted that glucose does not co-transport water - rather it is the now increased relative concentration of Na+ across the intestinal wall which pulls water through after it.

Several other molecules apart from glucose have a similar capacity to co-transport Na+ including

- amino acids (e.g. glycine)
- dipeptides
- tripeptides

The absorption of these molecules may occur independently of each other at different sites - thus their effect can be additive. Research is currently being carried on to utilize these additive effects to develop a multi-component "Super ORS".

Starch is metabolised in the intestine to glucose and therefore it has the same properties of enhancing sodium absorption, however it has an added advantage that it has less osmotic effect, which would act to pull water back into the lumen of the intestine.

Oral Rehydration Therapy (Ort)

Acute diarrhoea normally only lasts a few days. ORT does not stop the diarrhoea, but it replaces the lost fluids and essential salts thus preventing or treating dehydration and reducing the danger.

The glucose contained in ORS solution enables the intestine to absorb the fluid

and the salts more efficiently.

ORT alone is an effective treatment for 90-95% of patients suffering from acute watery diarrhoea, regardless of cause. This makes intravenous drip therapy unnecessary in all but the most severe cases.

The Composition of Ors, and findings in people.

In deciding the optimal composition of an oral rehydration solution the following considerations must apply:

Sodium - losses of sodium in the stool range from 50-60 meg/l to well over 100 meg/l in cholera and in fact total body depletion of sodium nay be higher than stool losses alone indicate. For this reason a Na+ concentration of 90 meg/l is considered an optimal figure for replenishing Na+ in dehydration from diarrhoea caused by any aetiology and in all age groups from neonates to adults.

For some years there was controversy over optimum concentration of sodium in oral rehydration fluids, which stemmed from the fact that in the early days of its use, particularly in USA, causes of hypernatraemia (excess sodium) occurred fairly frequently in infants given oral rehydration therapy.

The apparently obvious answer was to assume that the sodium concentration in the oral rehydration fluid used was too high and to reduce it (even to as low as 25 or 30 meg/l). Unfortunately, the apparently obvious was not the correct answer - actually nearly all these children were being given high- solute infant formula which tended to make them hypernatraemic to start with and the oral rehydration solution used then contained excess glucose - up to 8% - which was added to provide extra nutritive calories. Unfortunately, the excess glucose cause osmotic diarrhoea which precipitated acute hypernatraemia in these children.

The less obvious but correct answer was to reduce the glucose content not the sodium - we now recognize that the sodium and glucose should be in a 1:1 ratio in terms of molarity.

Experience has now shown that even hypernatraemic neonates with dehydration can be successfully rehydrated and made normonatraemic using the standard WHO / UNICEF ORS formula (with 90 meg/l Na+) when the water intake is sufficient to ensure normal kidney function and hence physiological regulation of the sodium concentration in the plasma.

Although ORS with a sodium content of around 50 meq/l is sufficient for maintenance of hydration of a normally will-nourished child with diarrhoea it would be inadequate for rehydration of a patient with a secretary diarrhoea (e.g., cholera) losing considerable sodium in the stool.

Glucose should be close to equivalent with the Na+ content - it is 111 mmol/l in the WHO / UNICEF formula, which happens to be exactly 2%. It should be noted that if glucose is present in excess of 3% it will cause further losses of water through osmotic effects, this would also upset the electrolyte balance, sine increased water losses will result in hypernatraemia.

Potassium. Although 98% of the body's potassium is held within the cells repeated diarrhoeal attacks over a period of time will cause a chronic loss of potassium - this results in muscular weakness, lethargy and anorexia. The typical distended abdomen of a chronically malnourished child is caused by loss of muscle tone in the abdominal wall largely due to chronic depletion of potassium. The kidneys are unable to conserve potassium as they do sodium, and there is a continuous obligatory loss of potassium of about 10 mmol daily in the urine, in addition to the larger losses in the stool.

Potassium is not involved in any way in the sodium/glucose co-transport mechanism and is absorbed passively. Restoration of potassium levels is therefore achieved more slowly than sodium and water restoration. A potassium concentration of 20 mmol/l is considered optimal for the purpose.

Simple mixtures of sugar, salt and water or starch, salt and water contain no potassium and cannot restore potassium depletion - hence these mixtures are an "incomplete" formula and further potassium supplementation is definitely necessary for a child who suffers repeated attacks of diarrhoea.

A potassium-rich diet including e.g. bananas or coconut water can be helpful but an ORS solution containing potassium is therapeutically more effective - in order to produce a significant effect it is necessary to provide potassium-rich foods in reasonable large quantities over a period of time.

Restoring a potassium deficit will stimulate the appetite and activity of the patient. If additional food is provided over several weeks an increase in weight gain will occur and the status of the animal's health will improve markedly.

Correcting Acidosis

Electrolyte imbalance and fluid loss also causes metabolic acidosis. These effects are more critical in the case of infants, as their renal function is not fully developed and they have a large surface area in ratio to body weight and a higher metabolic rate. Acidosis is corrected by the addition of bicarbonate (or another base such as citrate) to the ORS formula.

How Does Ort Work?

The causative pathogens of diarrhoeal disease (which are very numerous) in some cases not only produce the secretion of water and sodium but also damage the intestinal wall. The normal healthy intestine is covered on its inner surface with very numerous tiny hairs, or villi, the surface cells of which are involved in the absorption of metabolites from ingested food. There is a

difference between the cells of the tips of the villi and the cells of the base in their absorptive functions.

Pathogens, e.g., rotavirus, may strip the tips of the villi from large patches of the intestinal wall thus decreasing the surface area and decreasing by more than 50% the specific absorptive capacities of the intestine. The result is malabsorption which can cause malnutrition - most especially in an infant already nutritionally compromised by repeated previous attacks of diarrhoea.

Withholding food, even for one or two days, greatly exacerbates the malnutrition; this coupled with anorexia, caused partly by chronic potassium depletion, causes a vicious circle, i.e. diarrhoea causing malnutrition and malnutrition causing ever more frequent and severe diarrhoea. It is this diarrhoea/malnutrition cycle rather than acute dehydration that causes almost half of the five million deaths a year in under five year old children that are associated with diarrhoeal disease. Oral rehydration therapy (ORT), using a simple, inexpensive, glucose and electrolyte solution promoted by the World Health Organization (WHO) has reduced the number of deaths from dehydration due to diarrhoea in the human population by about a million per year.

Oral rehydration takes advantage of glucose-coupled sodium transport, a process for sodium absorption which remains relatively intact in infective diarrhoea due to viruses or to enteropathogenic bacteria, whether invasive or enterotoxigenic. Glucose enhances sodium, and secondarily, water transport across the mucosa of the upper intestine. For optimal absorption, the composition of the rehydration solution is critical. The amount of fluid absorbed depends on three factors: the concentration of sodium, the concentration of glucose and the osmolality of the luminal fluid. Maximal water uptake occurs with a sodium concentration from 40 to 90 mmol/L, a glucose concentration from 110 to 140 mmol/L (2.0 to 2.5 g/100 mL) and an osmolality of about 290 mOsm/L, the osmolality of body fluids. Increasing the sodium beyond 90 mmol/L may result in hypernatremia; increasing the glucose concentration beyond 200 mOsm/L, by increasing the osmolality of the solution, may result in a net loss of water. CHO to Na ratio should not exceed 2:1 in these solutions. Rehydration can be accomplished using solutions with higher sodium, i.e., 75-90 mmol/L. These are termed **rehydration solutions** (ORS). Prophylaxis of dehydration and maintenance involve solutions with 45-60 mmol/L of sodium. These are termed **maintenance solutions.** High sodium rehydrating solutions used to treat acute dehydration may be used for maintenance by giving the solution alternately on a 1-to-1 basis with a no-sodium or low-sodium fluid such as water, low CHO fluids, or breast milk. The high sodium ORS should not be used as the sole fluid intake for maintenance of hydration. Fruit juices and pop are not efficacious because of their high carbohydrate concentration, osmolality and the inadequate sodium concentration. Individualized dietary management of the patient during acute diarrhoea is the key and should be emphasised.

TABLE 3: Some commonly available Oral rehydration solutions and their components

Component	Rehydration			Maintenance		
	WHO	Rapolyte	Rehydralyte	Gastrolyte	Lytren	Pedialyte
Sodium (mmol/L)	90	90	75	60	50	45
Potassium (mmol/L)	20	20	20	20	25	20
Chloride (mmol/L)	80	60	65	60	45	35
Bicarbonate (mmol/L)	30	30				
Citrate (mmol/L)			30	30	30	30
Glucose (g/L)	20	20	25	20	20	25

Oral rehydration and maintenance solutions presently in use, although effective in rehydration, do not decrease stool volume because of the relatively high osmolality of the glucose which they contain. The challenge, therefore, is to provide adequate glucose to the sodium pump without increasing the osmolality of the rehydration solution.

This has been done successfully by substituting short chain glucose polymers (starch) from rice and other cereals for glucose in the oral rehydration mixture. In field trials in developing countries, ORS containing glucose polymers, primarily from rice and corn, were found not only to be as effective in correcting dehydration as glucose-based ORS, but also to offer the additional advantage of reducing the amount and duration of diarrhoea by 30%, thereby reducing morbidity and costs of treatment and increasing acceptability. Defined short-chained glucose polymers from rice may also be safe and effective in the treatment of acute diarrhoea. Wapnir et al found that a solution containing 30 g/L of rice syrup solids (180 mOsm/L) resulted in 40% more water absorption than a similar solution which contained 20 g/L of glucose (230 mOsm/L). A clinical study with solutions containing rice-syrup solids confirmed their efficacy in the rehydration of infants with acute diarrhoea. Further, such solutions decreased stool output, and promoted greater absorption and retention of fluid and electrolytes than did a glucose-based solution.

Amino acids have also been suggested as additives to ORS. The addition of alanine alone to the WHO oral rehydration solution (ORS) was not found to give additional benefits. However, Khin-Maung-U and Greenough found that alanine, added to a glucose polymer-based ORS, decreased the amounts of stool by a further 10% to 40%. Nevertheless, these are not currently recommended by WHO. Rice-based corn and lentil-based oral rehydration solutions have been extensively tested and may eventually be made available.

Along with improved oral rehydration solutions have come advances in the field of **early refeeding.** Fasting has been shown to prolong diarrhoea. This may be due to undernutrition of the bowel mucosa which delays the replacement of mucosal cells destroyed by the infection. Early refeeding with a lactose-containing formula is usually well tolerated and should commence 6-12 hours into therapy.

General Comments, Ort Protocol and Recommendations

Vomiting is not a contraindication to ORT. ORS should be given slowly but steadily to minimize vomiting. Fluids may be administered by nasogastric tube if required. The animal's clinical condition should be frequently assessed. An infant should never be kept on ORS fluid alone for more than 24 hours. Early refeeding should begin within 6 hours. A full diet should be reinstituted within 24 to 48 hours, if possible.

There are certain contraindications to the use of ORT

- Protracted vomiting despite small, frequent feedings
- Worsening diarrhoea and an inability to keep up with losses
- Stupor or coma
- Intestinal ileus.

TABLE 4: Simplified ORT protocol in mild to moderate rehydration

Assessment of dehydration	0-5%	5-10%
1st hour	20 mL/kg/hr	20 mL/kg/hr
For next 6-8 hours	10 mL/kg/hr administered evenly	15-20 mL/kg/hr
Reassessment at 4-hour intervals		
8-24 hrs	*Ad libitum* ORT solution Early refeeding	idem
> 24 hrs	Delayed refeeding only if severe vomiting	idem
There are many different equations for calculating administration rates in oral rehydration. ORT may be given in amounts equal to fluids calculated for intravenous administration. Alternately, fluids may be delivered by nasogastric tube		

RECOMMENDATIONS

- Dehydration accompanying infantile gastroenteritis should be treated with early oral rehydration and early refeeding strategies.
- Infants with gastroenteritis should be offered maintenance solution to prevent dehydration.
- Antidiarrheal drugs, antibiotics and antiemetic therapy are rarely indicated in gastroenteritis in juveniles and should be discouraged.
- Home-made oral rehydration solutions are discouraged since serious errors in formulation have occurred.
- Infants with mild to moderate dehydration should be treated under medical supervision with ORT in preference to intravenous rehydration.
- Infants with severe dehydration should initially be treated with intravenous or intraosseous rehydration.
- Early refeeding should commence as soon as vomiting has resolved, approximately 6-12 hours.
- Non-lactose containing formulae or milks may be used if diarrhoea and abdominal cramps persist beyond expected 5- to 7-day course suggesting clinical lactose intolerance.
- Further initiatives to encourage ORT use by patients and professionals should be developed.

PARENTERAL FLUID THERAPY

Parenteral Fluid Therapy Routes (Also See Section 1)

Subcutaneous

This is convenient for MAINTENANCE fluids. However it is inefficient in extreme dehydration because the intense vasoconstriction reduces absorption of the administered fluid. Only isotonic solutions should be given. SC administration of 5% dextrose should be avoided because equilibration of ECF with a pool of electrolyte free solution may result in aggravation of electrolyte imbalance.

Intraperitoneal

An easy method of fluid administration with a large reservoir for fluid introduction. However, fluid uptake is uncontrolled. The pH of the fluid administered may cause a chemical peritonitis and a peritoneal sepsis may result from a defective aseptic technique. If solutions are too cold, emesis or shock may develop.

Intraosseus

Can be used in any animal where a bone marrow needle can be placed and venous access is compromised. **This should only be conducted under anaesthesia, as it is extremely painful.**

Intravenous

When fluid loss is sudden (haemorrhage) or extensive, the IV route must be used. Catheters increase the efficiency and ease of IV fluid administration, (and blood sample collection), and reduce stress and pain to the animal. The saphenous vein provides an excellent, easily accessible site to place a catheter in monkeys, and this or the cephalic vein can be used in baby chimps.

Under visual guidance, or by palpating the vein with a gloved fingertip, the needle should be inserted about 1mm medial to the vein at an acute angle to penetrate it. To avoid haemolytic reactions and rapid coagulation, the use of wide lumen needles (0.9mm) and regular heparin flushes are recommended to maintain patency.

FORMULATING A PARENTERAL FLUID THERAPY PLAN

Replacement

Fluid and electrolyte deficit is most commonly due to vomiting, diarrhoea, haemorrhage or urinary loss. Fluid for replacement should therefore contain electrolytes that are approximately the same concentration as that lost. This is usually LACTATED RINGERS SOLUTION (HARTMANN'S). This will often need supplementation with 20mEq/L of KCl to add the required potassium.

Exceptions to LRS administration include

- Pulmonary and cerebral oedema
- Ruptured bladder (use 0% saline)
- Congestive heart failure (use 0.45% saline + 2.5% dextrose OR ½ strength LRS + 2.5% dextrose.

Maintenance

Insensible losses of fluids and electrolytes (GIT, UT, lungs, skin) contain roughly half the salt concentration found in serum or LRS, and about 4-5 times

the potassium – that is – potassium MUST be added to fluids to replace this loss – thus

LRS + 5% dextrose + KCl = ½ strength LRS + 2.5% dextrose and 20mEq/L KCl is an adequate maintenance solution.

Rate of Fluid Administration

The rate of fluid administration is determined by the rate and magnitude of the fluid loss. Rapid or extensive fluid loss requires rapid replacement. Provided congestive heart failure or anuric renal failure are not present fluid can be given as rapidly as 60-90mL/kg/hr. When fluid is administered this rapidly, ALWAYS assess the patient at 15 minute intervals to determine if the rate is too fast and fluid overload is occurring (see monitoring section below).

In chronic disease, fluid does not need to be replaced as rapidly. The replacement volume can be added to the daily fluid requirement and administered to the patient over 24 hours.

Fluid administration (LRS) at 5-10mL/kg/hr should be considered in any patient undergoing anaesthesia and surgery for periods of greater than 30 minutes. This is to help reduce the effect of cardiovascular compromise that occurs with all anaesthetic techniques and to counteract the major evaporative losses that occur when the chest or abdomen is open during surgery.

Amount of Fluid to Administer

Replacement volume

The replacement volume is determined by the estimated degree of dehydration (i.e. the amount of fluid lost by the patient).

E.g. 10kg chimp juvenile 10% dehydrated = 10 x 0.1 = 1. i.e., 1L of LRS is required for the replacement volume.

Maintenance fluids

A maintenance volume of fluid must be administered during any period that a patient is not taking in oral fluids. This accounts for obligatory fluid loss through the urinary tract, GIT, skin and lungs. Maintenance fluid requirements are **50-70mL/kg/24hrs.** This fluid must be administered in addition to the replacement volume

Ongoing Losses

Account of continued loss through vomiting, diarrhoea, urinary tract, large wounds, drains or peritoneal loss must be taken. An estimate is made of the volume lost and this is added to the replacement and maintenance volumes of

fluid administered over a 24 hour period. During surgery, 3mL of LRS should be administered for every mL of blood lost.

Monitoring Fluid Therapy

When fluid is administered rapidly (30-90 mL/kg/hr) the patient should be monitored every 15 minutes to ensure that volume overload or haemodilution has not occurred. Examination should include auscultation of the chest, paying particular attention to the lungs for an increase in interstitial/ alveolar sounds that may indicate the onset of pulmonary oedema. PCV and TP should be monitored to assess haemodilution.

During more chronic administration of fluid a physical examination should be carried out twice daily. This should include assessment of skin turgor, thoracic auscultation, PCV, TP and weight. An estimate of urine output should be made. Normal output is about 1-2mL/kg/kr.

Signs of fluid overload include serous nasal discharge, restlessness, cough, dyspnoea, pulmonary oedema, ascities, polyuria, exophthalmos, vomiting, diarrhoea.

If an IV catheter is in place, the catheter site should be checked daily for signs of redness and swelling. Other signs of sepsis e.g., increase in temperature should also be monitored. The catheter site should be changed every 72 hours even if there is no sign of infection present. When not in use the catheter should be flushed with 5 units/mL of 0.9% saline (heparinised saline). The catheter should be removed as soon as fluid therapy is no longer necessary to reduce the potential for sepsis.

A Theoretical Example

1. CALCULATE VOLUME OF FLUID REPLACEMENT REQUIRED

- Weigh and/or estimate weight of patient prior to illness
- Estimate degree of dehydration
- Calculate replacement volume required.

20kg chimpanzee that appears 8% dehydrated due to 10 days diarrhoea, unresponsive to ORT.

20x0.08 = 1.6 = 1.6L of LRS to replace the deficit

2. DECIDE UPON ANY ADDITIONAL POTASSIUM

If serum potassium concentration cannot be determined, use 20mEq/L of LRS

3. DECIDE UPON REPLACEMENT FLUID ADMINISTRATION RATE

- Decide over what time period you wish to give the LRS
- Calculate the rate of fluid administration from this

- Determine if potassium administration will be too fast (i.e. MUST be less than 0.5mEq/kg/hr to prevent hyperkalaemia.

Thus – You decide to give the fluid over 4 hours = 1.6L given over 4 hours = 400mL LRS/ hr = 8 mEq potassium/hr. The maximum potassium is 0.5mEq/kg/hr (so in this case 10). Therefore the replacement LRS can be given this rapidly without hyperkalaemia occurring.

4. CALCULATE MAINTENANCE FLUID VOLUME AND RATE OF ADMINISTRATION

- Calculate maintenance fluid volume.
  - 20kg x 50mL/kg/24h = 1000mL/24hr.
- Calculate maintenance fluid rate – over the first 4 hours we are administering the replacement fluid therefore we have 20 hours remaining over which to give the maintenance volume = 1000mL/ 20 hours = 50mL/hr.

We could, for example, administer the fluid over a 10 hour period then flush the catheter with heparinised saline until the next day to continue the next 24hr maintenance fluid requirements. Thus: 1000mL /10hr = 100mL/hr.

5. ESTIMATE ONGOING LOSSES

Record estimated amount of ongoing losses through vomiting, diarrhoea etc and add this to today's fluid as the patient loses the fluid or add the "extra" losses up over a 24 hour period and add them to the maintenance fluids for the next day.

6. DECIDE UPON DURATION OF PARENTERAL FLUID THERAPY.

Parenteral administration of fluid should continue until the patient can take in sufficient fluid orally to cover maintenance requirements. If a young primate is strong enough to remove an IV line, it can imply that it's strong enough to begin accepting ORT, if dehydration is still a problem.

PARENTERAL FLUIDS AND ADDITIVES OF USE IN PRIMATES

Blood and Blood Substitutes

Blood is of course the first choice for major blood loss, but unless a large healthy adult and proper facilities exists, this is unlikely to be used in the sanctuary setting. A blood substitute product is on sale in Japan but worries about long term effects means that it hasn't been licensed anywhere else.

Colloids

- These stay in the blood vessels where they maintain blood volume

- **Fresh frozen plasma** – unlikely to be available
- **Hydroxyethyl starch solutions** – Stable, long plasma half life (about 8 hours), long shelf life – ideal but VERY expensive.

Crystalloids

- These move rapidly out of the blood vessels into the extracellular fluid (ECF), but can still be useful to expand the blood volume in an emergency.
- **Sodium Chloride 0.9% solution (normal saline)** – Distributed throughout the ECF. Its lack of bicarbonate or a precursor tends to lower the pH, but in acidosis, increased blood volume may improve kidney blood flow and thus kidney regulation of pH leading to a reduced acidosis. Long term use will require extra potassium
- **Dextrose 5% solution** – Used as a means of supplying water (the dextrose is quickly metabolised). Distributed throughout the body water. DO NOT give subcutaneously.
- **Ringer solution** – Similar to normal saline but with some potassium. Also tends to lower the pH
- **Lactated Ringers Solution – LRS (Hartmann's solution)** – Commonly used for ECF replacement is it contains lactate (metabolised to bicarbonate). Will tend to raise the pH

Electrolyte additives

Potassium chloride solution – Comes in several strengths which **must be diluted before use**. They are usually mixed into a bag of crystalloid. Note that injecting potassium into a bag is NOT the same thing as mixing it with the bag's contents – a bolus of potassium will rapidly stop the heart. Longer term fluid therapy (> 12 hours) usually requires potassium supplementation.

Hyperkalaemia can be treated by

- Correcting acidosis
- Giving insulin in 5% dextrose to promote uptake of potassium by cells
- Giving calcium borogluconate to oppose the effects of the potassium

Sodium bicarbonate 8.4% solution **– Used to correct blood acidosis. This concentration is used because it contains 1mmol/mL which makes the sums easier.** It must be mixed with other solutions before use. **Normal saline is the fluid usually used – it is incompatible with many other solutions and most drugs. Bicarbonate administration is calculated to replace the circulating deficit ONLY, since correcting the acidosis entirely will lower plasma potassium levels. A blood gas sample (arterial sample) is taken and the amount of bicarbonate required is obtained by multiplying the base excess (a negative number in acidosis!) by the blood volume. Once this amount of bicarbonate has been infused, the base excess is checked again**

3.15 DRUGS FORMULARY FOR PRIMATES & PRIMATE SANCTUARIES

W.Boardman, E.Dubois, J.Fielder Review by Nonee Magre.

ANTI-BACTERIALS important drugs in bold.

GENERIC NAME	TRADE	DOSE	FREQ	COMMENTS
Furazolidone		5mg/kg PO	QID	
Gentamicin	*Genta*	2.4mg/kg	BID	
Amikacin	*Amikin*	5mg/kg IM	TID	
Isoniazid		15mg/kg PO	SID	For mycobacteriosis with ethambutol and rifampicin
Amidostaline	*Gabbroral*	20-40mg/kg	SID	Useful for salmonella and shigella and for enteric protozoa
Kanamycin	*Kantrex*	7.5mg/kg IM	BID	
Amoxicillin	*Amoxil*	**7-15mg/kg SC, IM, PO**	**BID**	**Gr + spectrum. Minor wounds, oral cavity problems**
Lincomycin	*Lincocin*	5-10mg/kg IM	BID	
Amoxicillin+Clavulanic acid	*Augmentin, Synulox*	**10-15mg/kg SC, IM, PO**	**BID**	**Gr+ with some Gr - spectrum. Respiratory, skin, urinary tract infections. May cause diarrhoea /GI upset. Can use up to 50mg/kg BID in severe infections**
Methicillin sodium		50mg/kg IM	BID	
Metronidazole	*Flagyl*	50mg/kg PO	SID-BID	Respiratory tract anaerobic infection suspected Hepatitis, Gastrointestinal upset/enteritis (also for protozoa.)Tastes very bad so very difficult to give
Ampicillin		50mg/kg SC,IV	TID	
Azithromycin	*Zithromax*	40mg/kg day 1 and then 20mg/kg for 5d	SID	
Neomycin		10mg/kg PO	BID	
Penicillin G benzathine	*Ancef*	20,000 iu/kg SC, IM	SID	
Cefazolin		20mg/kg SC,IM	TID	
Penicillin G potassi		7,000-14,000 IM or IV slowly	qid	
Cefixime		10mg/kg PO	SID	Otitis interna, Strep pneumonia, UTI. Resistant pseudomonas, Listeria, Staph and anaerobes
oxy				
Cefotaxime	*Claforan*	20mg/kg IM	TID	
Penicillin G procaine	*Fortan*	50,000iu/kg SC	SID	
Ceftiofur		90mg/kg IM. IV	q6-8h	Broad spectrum gr+, anaerobes. Adequate blood levels if given every 24-48 hr
Ceftriaxone	*Rocephin*	50mg/kg IM	SID	Excellent penetration into the CSF for meningitis
Rifampin		22.2mg/kg PO	SID	For mycobateriosis with ethambutol and isoniazid
Cephalexin	*Keflex, Ceporex*	25-40mg/kg PO	BID	Gr + broad spectrum. Respiratory, skin, urinary tract infections
Streptomycin		2.5-5mg/kg SC, IM	BID	
Cloxacillin	*Orbenil*	20mg/kg PO	BID	
Sulphasalazine		30mg/kg PO	BID	
Chloramphenicol succinate	*Chlormycetin*	25-50mg/ kg IM,PO	BID-QID	Excellent broadspectrum antibiotic - possibly drug of choice for gorillas
Ciproflaxacin	*Cipro, Ciproxin*	**10-20 mg/kg PO**	**BID**	**Can cause arthropathy in juveniles**
Tetracycline	*Terramycin*	25mg/kg PO, IM, IV	BID	
Clarithromycin		250mg PO	SID-BID	Human dose. Better availability with fewer GI signs than erythromycin. Very broad spectrum
Clindamycin	*Dalacin, Antirobe*	5.5-11mg/kg IM, PO	BID	Use with care when PO: can cause clostridial overgrowth and colitis. Give protexin
Trimethoprim/Sulphadiazine	*Septrin, Unitrim*	30-50mg/kg IM,SC,PO (total dose)	SID	Broad spectrum,Gr- with some Gr +. Gastrointestinal, respiratory, skin, urinary tract infections
Doxycycline	*Vibramycin*	5mg/kg PO for 1st 24 hrs & then 2.5mg/kg	BID	
Tylosin	*Tylan*	2mg/kg SC, IM	SID	
Enrofloxacin	*Baytril*	5mg/kg SC, IM or PO	SID-BID	Broad spectrum, esp Gr – Respiratory or gastrointestinal infections, any severe infection. Bad taste so difficult to give PO. Can cause arthropathy in juveniles
Vancomycin		20mg/kg IV, IM	BID	
Erythromycin	*Erythro-terra 100*	70 mg/kg. PO or 5mg/kg IM	BID	Respiratory infections or campylobacter diarrhoea
Ethambutol		22mg/kg PO	SID	For mycobacteriosis with isozianid and rifampicin

ANTIFUNGALS

Generic Name	Trade Name	Dose Rate	Route	Frequency	Comments
Amphotericin B	Fungizone Fungilin	0.25mg-1mg kg	IV slow	SID	
Fluconazole	Diflucan 50mg/cap	1mg/kg for tinea for 2-4 weeks(up to 6 weeks)	PO	BID	
Flucytosine	Ancotil 10mg/ml	50mg/kg for 7 days	IV	QID	
Griseofulvin	Grisovin 125mg/tab	5mg/kg	PO	BID	
Itraconazole	Sporanox 100mg/cap	3-4mg/kg	PO	SID or BID	
Ketoconazole	Nizoral 200mg/tab	3-5mg/kg	PO	BID	
Lufenuron	Program	60mg/kg with fat	PO	ONCE Repeat after 3 wks	Systemic and generalised cutaneous mycoses
Nystatin	Nystatin	7000 iu/kg	PO	QID	
Terbinifine	Lamisil 250mg/tab	3-4mg/kg for up to 2-6 weeks	PO	SID	

ANTIVIRALS

Generic Name	Trade Name	Dose Rate	Route	Frequency	Comments
Valaciclovir	Valtrex	15mg/kg	PO	TID	For herpes
Acyclovir	Aciclovir Zovirax	10-20mg/kg 5-10mg/kg	PO IV	QID TID	For herpes
Famciclovir	Famvir	3-4mg/kg for 7 days 5-6mg/gk for 7 days 10-12mg/kg for 7days	PO	TID BID SID	For herpes

ANTI PROTOZOALS

GENERIC NAME	TRADE	DOSE	FREQ	COMMENTS
Albendazole		10mg/kg PO	Once	Repeat in 2 weeks
Aminosidine	***Gabbroral***	15mg/kg	SID for 5-6 days	for Entamoeba, giardiasis, trichomoniasis
Artenam	***Artemether***	3.2mg/kg PO, IM on day 1 and 1.6mg/kg for the next 4 days	SID	For malaria – not trialled
Bunamidine		25-100mg/kg, PO	once	For cestodes
Chloroquine		10mg/kg PO,IM once then 5mg/kg 6 hours later, then 5mg/kg q24 for 2 days		For malaria. Use with primaquine
Clindamycin		12.5-25mg/kg	BID	For toxoplasmosis
Doxycycline		20mg/kg PO	TID for 5-10 days	For Balantidium coli
Fenbendazole	***Panacur***	50mg/kg	once or for 3 days	for nematodes
Furazolidone		10mg/kg PO	BID for 3-5 days	For protozoa
Ivermectin	***Ivomec***	0.2-0.4mg/kg	once	For nematodes and mites Repeat in 3 weeks
Mebendazole	***Vermox***	25mg/kg PO	SID for 3 days	For nematodes
Metronidazole	***Flagyl***	30-50mg/kg PO	BID for 10days	For protozoa
Niclosamide		100mg/kg, PO	once	For cestodes
Oxytetracycline	***Terramycin***	1500mg/kg/day IV by continuous infusion		For gorillas with Balantidium coli
Paromomycin	***Humatin***	12.5-15mg/kg PO	BID for 3-10 days	For Balantidium coli and entamoeba histolytica
Praziquantel	***Droncit***	40mg/kg PO, IM, SC 15-20mg/kg PO,IM,SC	Once once	For trematodes For cestodes
Primaquine		0.3mg/kg PO	SID for 14 days	For malaria
Pyrantel pamoate	***Combantrin,Antiminth, Danmint***	11mg/kg PO	once or SID for 3 days	For nematodes

	h			
Pyrimethamine	***Darapri m***	2mg/kg PO	SID for 3d and then1mg/kg for 4 weeks	For toxoplasmosis
Tetracycline		15mg/kg PO	QID for 14 days	For Balantidium coli
Tinidazole	***Fasigyn Tiniba***	30-50mg/kg PO	Once SID for 3-5 days	For Giardia For Balantidium coli and Entamoeba
Trimethoprim/Sulp ha	***Septrin***	30mg/kg PO	QID for 14 days	for Pneumocystis carinii

ANTIMALARIAL DRUGS

Generic Name	Trade Name	Dose Rate
Artemether	Artenam	3.2mg/kg PO, IM on day 1 and 1.6mg/kg for the next 4 days
Mefloquine	Lariam 250mg	¼ table single dose PO
Halofantrine	Halfan 100mg/5ml susp	24mg/kg in 3 doses at 6 hourly intervals
Chlorquine	Nivaquine 200mg or 5mg/ml susp	10mg/kg on day1 followed by 5mg/kg 6 hours later. 5mg/kg SID for 3 days
Quinine	Quinimax 100mg tab	25mg/kg SID for 7 days
Doxycycline		
	Malerone	

VITAMINS AND SUPPLEMENTS

Generic Name	Dosage	Route
Iron Dextran	10mg/kg	IM
Vit A, D_3 and E	100 000iu A 0.5-1.0ml 10 000iu D_3 20IU E	IM
Vit B complex	100mg B1 0.5-1.0ml 5mg B2 100mcg B12	IM
Vit C	5-10mg/kg	PO SID
Zinc	2.5mcg/day for 3 days	

DIURETICS

Drug	Tradename	Dose/Reason	Route	Frequency
Acetazolamide	Acetazolam, Dazamide, Diamox	2-5mg/kg for glaucoma	PO	TID
Furosemide	Furoside, Dimazon, Lasix	1-4mg/kg	PO,SC, IV, IM	SID-QID
Mannitol	Osmitrol Mannitol 20%	2g/kg for cerebral oedema, spinal cord trauma or acute glaucoma	IV slowly	

OPIOIDS, CORTICOSTEROIDS AND NON STEROIDAL ANTI INFLAMMATORIES:

Generic Name	Tradename	Dosage/Use	Route	Freq
Acetominaphen	***Tylenol*** ***Paracetamol***	10-20mg/kg	PO	QID
Adequan		2mg/kg q 3-5 days for 2-3 months	PO	See before
Aspirin		20mg/kg 10mg/kg	PO PO	BID Q4-6h
Buprenorphine	***Temgesic – 0.3mg/ml*** ***Buprenex***	0.02-0.05mg/kg	IM	BID
Butorphanol	***Torbugesic - 10mg/ml*** ***Stadol***	0.05-0.1mg/kg	IM	BID
Carprofen	***Rimadyl***	2-4mg/kg	PO	BID
Dexamethasone	***Axium, Dexate***	2-8mg/kg for **shock**	IV	once
Dexamethasone		0.25-1mg/kg as **anti-inflamm**	IM,PO	SID
Dexamethasone		2mg/kg for **cerebral oedema** and then 1mg/kg	IV SC	 TID
Fentanyl	***Sublimaze***	0.05-0.15mg/kg	IM	
Flunixin	***Finadyne*** ***Banamine***	0.5-2mg/kg	SC, IV, IM	BID to SID
Ibuprofen	***Brufen***	6-10 mg/kg 24mg/kg	PO PO	BID-TID SID
Ketoprofen	***Ketofen***	5mg/kg	IM	TID
Morphine		1-2mg/kg	PO, IV, SC, IM	Q4h
Naloxone	***Narcan***	0.01-0.05mg/kg	IM, IV, SQ	
Naproxen	***Naprosyn***	10mg/kg	PO	SID
Oxymorphone	***Numorphan***	0.025mg-0.15mg/kg	IM, IV, SC	BID
Pethidine	***Demerol***	1-2mg/kg	IM,IV,SC	QID
Prednisolone sodium succinate	***Soludeltacortef***	11-25mg/kg for **shock**	IV	
Prednisolone sodium succinate	***Soludeltacortef***	2-4mg/kg for **allergic bronchitis, asthma**	IV, IM	
Prednisolone		0.1-0.5mg/kg for **allergic reactions**	SC, IV	TID-

				QID
Prednisolone		1-2mg/kg for **immunosuppression** taper off over 2-3 months for immune skin diseases, SLE, rheumatoid arthritis, eosinophilic garstritis/enteritis	PO	BID
Triamcinolone	***Kenalog***	0.02-2.0mg/kg	IM	Once

MISCELLANEOUS DRUGS

Generic Name	Tradename	Dosage/Use	Freq
Apomorphine		0.08mg/kg IM,SC to **induce vomiting**	once
Bismuth	***Pepto bismol***	1ml/kg **intestinal protectant**	
Cimetidine	***Tagamet***	10mg/kg IM,PO for **gastric ulceration**	TID
Diphenhydramine	***Benadryl***	5mg/kg IM **Anti tussive**	
DMSA	***Chemet***	10mg/kg for **lead chelation** PO	Q8h for 5 days then q12h for 2wks
Heparin1000iu/ml		Saline 5 iu/ml saline Transfusion 400-600iu/100ml blood **Anticoagulant**	
Furosemide		2mg/kgPO **Diurectic**	As needed
Glipitide	***Glucotrol***	1.1mg/kg PO for **early stage or gestational diabetes mellitus**	Q24h
Loperamide	***Imodium***	0.04mg/kg PO **to reduce intestinal motility**	TID
Medroxy progesterone acetate	***Depoprovera MPA 10mg/tab***	2.5-3mg/kg IM 10mg/kg PO **to suppress oestrous**	Q3m
Metaclopromide	***Reglan*** ***Primperan***	1-2mg/kg IV drip over 24 hours as antiemetic 0.2-0.5mg/kg PO, SC	
Nandrolone		2.5mg/kg IM **Anabolic**	
Neostigmine		0.07-0.08mg/kg IM	
Oxytocin	***20iu/ml***	5-10iu IM for **uterine inertia**. May repeat in 30mins 5-10iu IM for **uterine prolapse** 2-5iu IM for **milk letdown**	 once
Pancuronium		0.04-0.1mg/kg	
Potassium		IV intake max 0.5mEq/kg/hour ORAL intake- 2-4meq/kg/day PO SQ intake 40meq/L dilute in fluids for SQ injection **Potassium loss through D and V**	
Ranitidine	***Zantac***	0.5mg/kg PO ***Anti ulcer***	BID
Xylasine	***Rompun***	0.5-1.0mg/kg IM,SC to induce vomiting	once

HORMONES

Generic Name	Tradename	Dosage	Route	Freq
ANDROGENS/ANABOLIC STEROIDS				
Ethylestrenol	Nandoral	Supportive treatment for renal failure	PO	SID

Nandrolone	Deca-Durabolin	5mg/kg for anabolic effects and bone marrow stimulant	IM	Q 7 d
Oxymethalone	Anadrol	1mg/kg for anabolic effects	PO	B I D
Methyl testosterone	Metandren	1-2mg/kg for anabolic effects	PO	S I D
OESTROGENS/PROGESTAGENS				
Medroxyprogesterone acetate	Depo provera	For the prevention of oestrous		

RESPIRATORY TRACT DRUGS

Types of Drugs	Trade Names	Uses
Benzonatate		suppresses cough reflex
Codeine		suppresses cough reflex and analgesic
Dextromorphan		suppresses cough reflex
Guiafenasin	Toplexil	reduces viscosity of secretions
Theophylline	Theophylline	bronchodilator
Acetaminophen`	Toplexil	analgesia and antiinflammatory
Pseudoephedrine HCL	Nussidex	produces vasoconstriction
Dexchlorpheniramine	Nussidex	anti –histamine
Carbocysteine	Medibronc	reduces viscosity of secretions
Diphenhydramine	Diphedryl	anti histamine
Bromohexine	Bisolvon	reduces secretions

EMERGENCY DRUGS

Generic Name	Tradename	Dosage	Route
Adrenaline		0.01mg/kg every 3-4 minutes	IV
Atropine		0.05-0.1mg/kg	IV/IM
Dexamethasone		2-4mg/kg **Shock**	IV
Diazepam	Valium	1mg/kg	IV
Dopamine		1-5mcg/kg/min	IV
Doxapram	Dopram	1-2mg/kg Infant 0.1ml SQ under tongue	IV
Frusemide	Lasix	1-4mg/kg	IV
Lignocaine		1-2mg/kg for ventricular fibrillation	IV
Mannitol		0.5g/kg **Cerebral oedema** 0.25-0.5g/kg **Diuresis**	IV
Potassium		20-40meq/L fluids – dose **NOT** to exceed 1meq/kg/min	IV
Prednisolone	Soludeltacortef	10mg/kg **Shock**	IV, IM
Sodium bicarbonate		1meq/kg IV bolus over 10-15 minutes **Acidosis**	IV

PSYCHOTIC ACTIVE DRUGS IN CHIMPANZEES.

Uses

- Reduction in anxiety
- Reduction in aggression
- To improve opportunities for integration

These are drug doses taken from British National Formulary 38, September 1999 and are human dose rates, based on 70kg body weight.

NB. Many of these drugs have not been used clinically – caution should be exercised.

PSYCHOTIC ACTIVE DRUGS - ORAL

Generic name	Tradename	Dose	Dose Rate
Zuclopenthixol dihydrochoride	***Clopixol***	10-15mg BID leading to 75mg BID. Usual maintenance is 20-50 mg	
Haloperidol	***Haloperidol*** ***Dozic*** ***Haldol*** ***Serenace***	1.5mg BID initially to 5mg BID	12.5-25mg/kg BID
Pimozide	***Orap***	2mg SID increase by 2-4mg each week until required affect. Usual dose range 2-20mg SID	
Trifluoperazine	***Trifluoperazine*** ***Stelazine***	2-3mg BID	

PSYCHOTIC ACTIVE DRUGS - INJECTABLE

Generic name	Tradename	Dose	Basic Guide	Duration of Action
Zuclopenthixol acetate	***Clopixol-Acuphase***	50-150mg IM; max 4 injections or 2 wks		2-3 days
Zuclopenthixol decanoate	***Clopixol or Clopixol conc***	100mg IM initially Followed after 7-28 days with 100-200mg. Followed by 200-400mg at intervals of 2-4 wks	200mg every 2 wks	Approx 2 wks
Fluphenazine decanoate	***Modecate or Modecate concentrate***	12.5mg IM initially. Followed after 7 days with 12.5-100mg Followed after 14 –35days with 12.5-100mg	25mg every 2 wks	Approx 2 wks
Haloperidol decanoate	***Haldol decanoate***	50mg initially every 4 wks Increase by 50mg every 2 wks to 300mg every 4 wks	100mg every 4 wks	Approx 4 wks
Perphenazine	***Trilafon***			

GASTROINTESTINAL TRACT DRUGS

Antacids/Adsorbents

Aluminium carbonate, hydroxide, phosphate
Calcium carbonate
Magnesium oxide
Simethicone
sodium bicarbonate

Combination products also available

Aludrox susp	(1+3)
Camalos tablets	(1+2+3)
Gavsicon	(1+3)
Gelusil	(1+3+4)
Maalox	(1+3)
Maalox Plus tabs	(1+3+4)
Riopan Plus tabs	(1+3+4)
Titralac tabs/susp	(2+4)

Antidiarrhoels

Attapulgite	Diasorb, Kaopectate advanced formula
Bismuth subsalicylate	Bismatrol, Peptobismol
Kaolin and pectate mixtures	kapoectin, kapectolin, Kaospen
Loperamide	Imodium
Activated charcoal	
Spasmolytics and analgesics	Buscopan Comp
Combination products	Kaopectate II caplets
	Maalox diarrhoea
	Pepto diarrhoea

Laxatives

Lactulose	Cephulac, Chronulac, Duphalac
Magnesium citrate	Citro-mag, Citronesia
Magnesium hydroxide	Milk of magnesia
Magnesium sulphate	Epsom salts
Mineral Oil	Paraffin oil

Antiemetics

Meclizine HCl	Ancolan, Antivert
Metoclopromide	Primperan, Reclomide, Reglan
Thiethylperazine maleate	Torecan

Anti Ulcer drugs

Cimetidine	Tagamet
Misoprostol	Cytotec
Sucralfate	Sulcrate, Ulcar susp

Induction of Vomiting:

Apomorphine	0.08mg/gk IM,SC
Syrup of Ipecacuana	1-2ml /kg PO
Xylasine	0.5-1.0mg/kg IM,SC

Gastric Lavage

Needs to be done within 1-2 hours of intoxication:
Patient needs to be unconscious or lightly anaesthetised

Insert tight fitting endotracheal tube to prevent aspiration of gastric contents
Stomach tube – large bore for egress of fluids
Stomach tube – small bore to ingress of fluids
Repeat until lavage runs clear – usually 5-10ml/kg water or saline solution
Administer activated charcoal 2g/kg
Remove stomach tubes
Leave endotracheal tube in place until animal is semiconscious

CARDIOVASCULAR SYSTEM DRUGS

Ionotropics

Digoxin
Digoxin, Lanoxin

Strengthens myocardial contraction
0.003-0.07mg/kg PO BID 5 days per week

Anti arrthymic Drugs

Propanolol (Inderal) ie ventriclar arrthymias

0.2mg/kg PO BID /TID
2.5-5mg PO BID/TID for angina/hypertension

Anti anginals

Diltiazem HCl (Cardizem, Dilatam)
Dilates coronary arteries, treatment of hypertension and angina pectoris

Nitroprusside (Nipride, Nitropress)

Vasodilator for acute congestive heart failure
1-10mcg/kg/min IV infusion

Antihypertensives

Diltiazem HCl (Cardizem, Dilatam)
Dilates coronary arteries, treatment of hypertension and angina pectoris
0.5-1.3mg/kg PO TID

Benazepril HCl (Lotensin)

Enalapril (Vasoctec)

10-40mg daily

Prazosin (Minipress)
1mg/15kg PO BID/TID

Use alone or in combination with diuretics

CNS DRUGS

Anticonvulsants

Clonazepam (Klonopin)
For atypical seizures, akinetic, and myoclonic seizures
0.01-0.03mg/kg PO in 2-3 individual doses

Diazepam (Valium)
Restraint 0.2-0.6mg/kg IV
Seizures 1-4mg/kg PO TID/QID
Status epilepticus 0.5-1.0mg/kg IV in increments of 5-10mg to effect

Pheonbarbital sodium
Seizures 1-2mg/kg PO BID initially up to 10mg/kg/day
Status epilepticus 3-30mg/kg IV to effect

Phenytoin (Dilantin)
Seizures 15-40mg/kg PO TID

Primidone (Mysoline)
Seizures 15-20mg/kg/day in divided doses BID/TID

Antidepressants

Clompiramine HCl (Anafranil)
Obsessive, compulsive disorder
0.5-2(max3) mg/kg divided in two doses

Fluoxetine HCl (Prozac)
Obsessive, compulsive disorder, depression
0.3-0.5mg/kg PO am, maybe given BID, morning and at noon. Max 1mg/kg/day

Imipramine HCl (Tofranil)
Depression, narcolepsy
0.5-1.0mg/kg PO BID/TID

Anti Anxiety
Busipirone HCl (Buspar)
Anxiety disorders, short term relief of anxiety
0.1mg/kg PO TID; dosage increase at 3 day interval in 0.1mg increments

Diazepam (Valium)
Anxiety 0.2-0.5mg/kg PO BID/TID

Hydroxyzine HCl (Atarax)

Oxazepam (Apo oxazepam, Serax, Serepax)
Severe anxiety
0.2-0.4mg/kg PO TID

TOPICAL DRUGS

Anti Infectives

Antiviral

Acyclovir (Zovirax) for herpes simplex genitalis and labialis

Fungistatic

Amphotericin B cream, lotion (Fungizone)
Clotrimazole cream, lotion (Mycelex)
Econazole cream (Spectarde)
Ketoconazole cream (Nizoral)
Nystatin cream, ointment (Mycostatin)
Sulfaconazole nitrate cream (Exelderm)

Bactericidal, bacteriostatic

Bacitracin ointment
Gentamicin ointment, cream
Neomycin cream, solution
Sulfadiazine (Flamzine) for second and third degree burns
Tetracycline/Gentian violet sprays

Antispetic/ Local Anaesthetics

Benzalkonium chloride and lidocaine 2.5 (Bactine)

Scabicides and Pediculicides

Lindane (Kwell and Scabene)
Permethrin (Elimite)

Pyrethrins (base, Bluegel, Pronto)

Topical Corticosteroids

Betamethasone (Alfatrex, Diprosene)
Dexamethasone (Decaderm, Decadron)
Fluociniolone (Fluocet, Synalar)
Hydrocortisone (Acticort, Cortril, Dermacort)
Triamcinolone (Kenalone, Traicet)

Miscellaneous

Hydrogen peroxide 20 vol(3%)
Povidone iodine solution (Betadine)
Salicyclic acid, malic acid, propionic acid (Otoderm, Oticlens) to deslough wounds

OPHTHALMIC DRUGS

Ophthalmic Anti infectives

Ointments include

- Bacitracin
- Choramphenicol
- Ciprofloxacin
- Gentamycin sulphate
- Idoxuridine
- Norflaxacin
- Polymixin B
- Silver nitrate 1%
- Sulphactemide Na
- Tetracycline
- Neomycin
- Tobramycin

Combination with corticosteroids

Hydrocortisone/betamethasone/dexamethasone commonly combined with antibitoucs and sulphonamides

Non Steroidal antiinflammatory

Diclofenac –sodium 0.1% (Voltaren Opht)

Antibiotic Combination Products

AK poly Bac – polymixin B and bacitracin
Cortisporin - poylmixin B, bacitracin, neomycin, hydrocortisone
Isopto Cetapred - sulphacetamide-Na, prednisolone
Maxitrol ointment - neomycin, polymixin B, dexamethasone
Mycitracin Opht - choramphenicol, polymixin B, hydrocortisone

Tobra dex - tobramycin,chlorobutanol, dexamethasone
Vasocidin Opt - sulphacetamide Na, prednisolone

Miotics

Ocusert pilo, Isopto carpine, Miocarpine – Pilocarpine

Mydriatics

Atropisol, Isopto atropine - Atropine sulphate
Mydriacyl - tropicamide

MISCELLANEOUS OPHTHALMICS

Flourescein Na strips fluor-I-strip, Flourescein Paper
Schirmer test
Lidocaine drops Noversin
Dorsolamide hydrochloride Trusopt (ocular hypertension, open angle glaucoma)
Maleat Timoptic sol, Timoptic XE (ocular hypertension, open angle glaucoma)
Cyclosporin 1-2

Otic Solutions

Solutions, suspensions, ointments. Combinations contain antibiotics, anti inflammatories, analgesics, antiparasitics, antmycotics and cleaning solutions

TREATMENT OF POISONING

Poison	Generic Drug	Tradename	Dose
Bleach	Aluminium hydroxide	Alucap, Alu hydroxide gel, Alu tabs	100-500mg PO
Coumarin anticoagulants i.e. rat bait	Vitamin K1	Mephyton Konakion	3-5mg/kg/day PO for 1-4 weeks
Iron	Deferoxamine	Desferal	10mg/kg IM q8h for 24 hours (not to exceed 80mg/kg in 24 hours)
Lead	Ca EDTA Dimercaprol Penicillamine	 BAL 10% in oil Cuprimine	Dilute to 3% or less for IV use 25-35mg slow IV over 1 hr, BID for 5-7 days 2-4mg/kg IM TID-QID 15-20mg/kg PO BID
Metaldehyde	Diazepam Pentobarbitone	Valium	2-5mg/kg IV slowly to contrail tremors To effect IV
Morphine or other opioids	Naloxone	Narcan	0.1mg/kg IV
Organophosphate	Atropine		0.2-0.5mg/kg ¼ IV remainder IM,SC
Snake bite	Antivenines specific or polyvalent		Use venom extractor Use specific antivenines or polyvalent Administer antihistamine Corticosteroids contra indicated

			Fluid therapy Antitetanus therapy Broadspectrum antibiotics
Strychnine	Pentobarbitone		To effect IV
Zinc	Ca EDTA Dimercaprol Penicillamine	 BAL 10% in oil Cuprimine	Dilute to 3% or less for IV use 25-35mg slow IV over 1 hr, BID for 5-7 days 2-4mg/kg IM TID-QID 15-20mg/kg PO BID

VACCINATIONS

Tetanus
Use human species tetanus toxoid
Give IM because SC deposition of aluminium adjuvants may cause sterile abscesses

Measles
Use Modified live vaccine; do not vaccinate pregnant animals

Vaccinate for Great Apes
1 - > 6months of age
2 – 13-15months of age
3. – 10-12 years of age

Poliovirus
Use modified live oral vaccine, shedding of the vaccine virus may occur

1. – 2months
2. – 4 months
3. – 18months
4. – 4-6years
5. – 14-16years

Adults
Every 2 months for three vaccinations

Hepatitis B
Two injections at one month intervals and then 3rd vaccination 6 months after 1st injection
0.5ml IM

Homemade Oral Rehydration Therapy
½ teaspoon of Potassium Chloride (KCl)
½ teaspoon of Sodium bicarbonate (NaHCO3)
½ teaspoon of Sodium chloride (NaCl)
2 tablespoons of Glucose **or** 4 tablespoons of sucrose
In 1 (one) litre of water

In the dry state it lasts indefinitely and made up, lasts 24 hours
Palatability maybe improved by adding small quantities of powdered orange juice

3.16 PRIMATE REPRODUCTION and CONTRACEPTION

S.Unwin W. Boardman and S. Walker (with information from the AZA-Contraception Advisory Group).

Part 1. Primate reproductive physiology and tips on monitoring reproductive cycles

CHIMPANZEE REPRODUCTION

Development	General	Wild	Captive
Female	Early oestrous swellings are usually small and irregular. Menarche (sign of first menstruation) occurs 1 –1.5 years after first swellings. Oestrous swellings do not reach maximum size until after menarche. Once swellings occur, they copulate frequently. May become pregnant for the first time from 4 months to 2 years after menarche.	First oestrous swelling 8.5 – 9.5 years. Menarche typically occurs at 11 years Pregnancy may occur from 11 years 4 months of age to 13 years.	First oestrous swellings 5.5 – 7.5 years. Menarche typically occurs 6.5 – 9.0 years. Pregnancy may occur from age 7 to 11.
Male	Mounting, thrusting and intromitting can occur as young as 2 years	Rapid testicular growth occurs between 9-10 years. Socially mature at 15 years	Rapid testicular growth occurs between 6-7 years. Adult weight and dentition occurs between 8 and 9 Need to be sociably capable of successful copulations which can occur between 11 and 12 years. Some aged 7-9 years have sired offspring

Oestrous Cycle

Duration usually last 34-37 days

Oestrous cycles continue through out life and have been observed in females of 45 years and older and females behaviour may change through out the cycle. Males increase time spent with a female when she is swollen. They are more likely to groom her and examine or manipulate her swelling. Copulation can occur at any stage in the oestrous cycle, including pregnancy and menstruation. Anovulatory swellings can occur during pregnancy. Copulation coincides with maximal swelling and peaks in the morning.

Cycle divided into 4 phases

Phase	Duration	Details
Pre-swelling phase	6-7 days	begins first day after the end of menstruation skin is quiescent
Swelling phase	17 –18 days	The perineum becomes increasingly swollen but may fluctuate during the day at maximal swelling (tumescence) it is free of wrinkles and shiny and last 6-7 days (out of the 17-18 days). Ovulation occurs on the last day of maximal swelling and can be predicted by labial occlusion of the medial surfaces of the labia minora pressing together so that the vagina appears as a slit.
Post-swelling phase	10 days	Swelling is rapidly lost over 4 days and then becomes quiescent
Menstruation phase	3 days	begins usually 6 – 12 days from the start of detumescence menstrual discharge duration and quantity can vary, sometimes it can be missed by observers.

Gestation

Gestation Period: 227 days ± 12 days = 32.5 weeks (31-34 weeks)

Signs of Pregnancy

- Initially signs maybe difficult to observe, because menstruation and oestrous swelling can continue for the first months of gestation. However, the swelling maybe smaller in size and less regular.
- Swellings can disappear by the end of the first trimester although in some females the swellings can continue throughout the gestation period.
- Morning sickness has been described (more common in primiparous females)
- Irregularity of appetite
- Mammary enlargement and self-manipulation and/or visual inspection of the nipples
- Distension of lower abdomen maybe noticed in the last few months.

Impending Parturition

Signs of Impending parturition

- Increased nipple manipulation
- Slight bulging of the perineal area
- Increase in frequency of urination and projectile like expulsion of the urine maybe seen several weeks before birth
- Females may taste urine and rub on nose one week before birth
- Some females become lethargic and lose appetite one week before birth

- Within 24 hours, the rump may appear concave and vaginal dilation can be seen.

Parturition

Labour is usually 30 – 40 minutes but can be up to 8 hours

- Females become restless and take various postures
- Small amounts of blood and amniotic fluid are seen
- Other chimps inspect the genital area
- Straining may be observed
- The female will often hold, catch or pull the neonate and then retrieve and cleans it immediately
- Placenta is often delivered within minutes and the mother will break the cord. The placenta and fluids are often consumed by the mother.
- Some neonates have to be assisted because they cannot cling well and will often nurse irregularly in the first five days.
- Females with prior exposure to other infants are more likely to show good maternal behaviour.
- Other chimps particularly females show intense interest in the offspring and will often touch it. Some females will often take the youngster to care for it. This is normal but abduction can occur which is not acceptable by the mother.

Resumption of Oestrous Swellings

- No activity occurs due to the presence of lactation
- Usually 14 months to 4 years
- Several swellings may occur before fertilisation may occur.

Interbirth Interval

- Wild → Average is 5 years 8 months
- Captive → 2 years 10 month s to 6 years 6 months have been recorded.

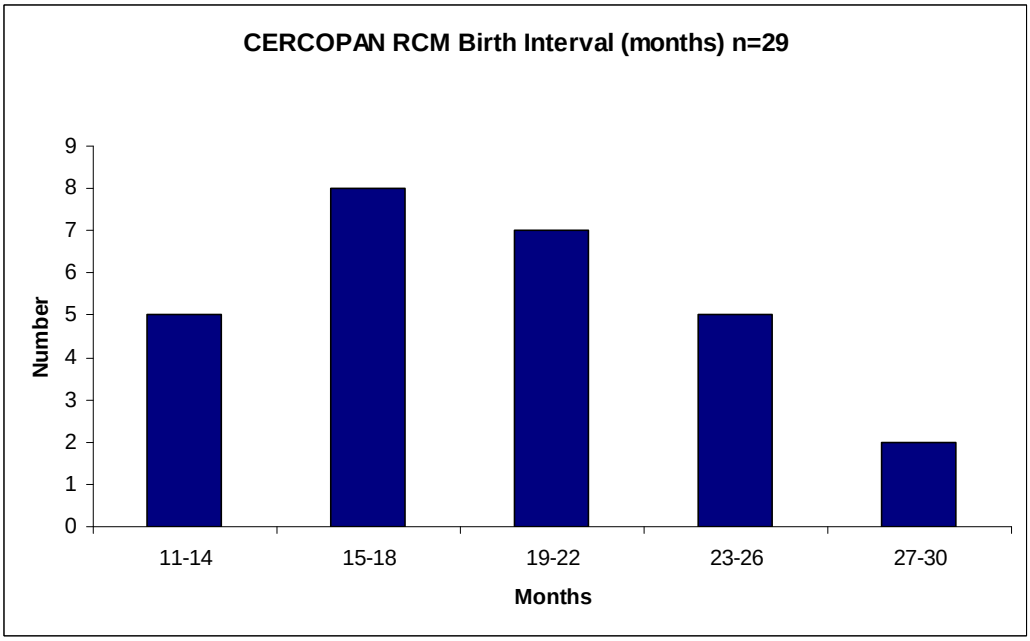
MANGABEY REPRODUCTION (courtesy of CERCOPAN)

Red Capped Mangabey Reproductive Data

Age at 1st Birth (months). Calculated from captive born individuals only as these are the only ones with accurate age.
n=7. Mean: 47.4. Std Dev: 12.7

Birth interval (months)
n=27. Mean: 19.1. Std Dev: 4.9 Range: 11-29

Average time to next birth after infant death (months) (n=6)
n=6. Mean: 10.2 Std Dev: 3.8. Range: 7-15



Measuring of Mangabey sexual swellings
Scale of 0-4

4	-	Being the highest
0	-	Not in season
2?	-	Means Onset of Swelling
2?	-	Dwindling or Reducing Swelling
? or?	-	Can also be determined by Redness of swelling.

NB: Pale swelling may mean reducing of oestrus

Part 2. Primate Contraception

REASONS FOR CONTRACEPTION

- Space issues – good management
- Prevent hybridization
- Temporary/ reversible while in captivity
- Do we know the relatedness of the individuals under our care? DNA Analysis can assist with this. If you don't have access to this, contraception is recommended in the interim until relatedness can be ascertained.

CONTRACEPTION OPTIONS

- Surgery – permanent, dangerous under field conditions
  - Male (social issues)
    - Vasectomy (occasionally reversible)
    - Castration
  - Female (degree of surgical difficulty)
    - Ovariectomy
    - Ovariohysterectomy
    - Tubal Ligation
- Husbandry (abstinence)
    - Separating animals – e.g. Bachelor groups
- Physical barriers - IUD's
- Chemical

See below for the latest AZA and EAZA recommendations for non human primates**.**

IT IS RECOMMENDED THAT WHATEVER OPTION A SANCTUARY CHOOSES, THEY SHOULD FEED THEIR INFORMATION ON CONTRACEPTION TYPE/ SUCCESS ETC INTO THE INTERNATIONAL ANIMAL CONTRACEPTION DATABASE.

As at 2009, the European contact for this database is Dr. Sue Walker, based at Chester Zoo UK (s.walker@chesterzoo.org).

Chemical Contraception

1. Synthetic progestins

General Problems
- Blocking ovulation, causing thickening of cervical mucous, slowing ovum transport, and/or interfering with implantation or fertilisation
- May still get follicle growth and enough oestrogen production to cause oestrus behaviour
- Ovulation may also occur, even though no pregnancy

Reversibility
- Confirmation on documentation that ovulatory cycles have resumed
- CIRCULTING hormone will be gone within days

- Reproductive history, age, health, thin, fat, partner fertility

Contra Indications

- Don't use implants in pregnant animals, due to possibility of prolonged gestation, stillbirth, or abortion. However, there are numerous anecdotal reports where an implant has been inadvertently inserted early in an unknown pregnancy, with no apparent effect on that pregnancy, and off spring being born normally.
- Have been prescribed for lactating women – considered generally safe for nursing infants
- Not recommended in juveniles – lack of data
- Probably cause weight gain in all species

Implant - Implanon

This is an implant which is inserted subcutaneously whilst the animal is under general anaesthesia. It contains the active ingredient etonogestrel (68mg/ implant), and is effective for up to 3years. It is a human product, and has been widely used in women in Europe and Australasia; however it is only recently gone on the market in the USA. Each implant costs ~€150.
Anecdotally this implant has used successfully in chimpanzees, mandrills and gorillas (Various zoo reports, author personal experience).

Implant latency to effectiveness

- Individual variation
- Threshold levels of the hormone in the blood by 1-3 days post IM and within 1 week post SC insertion.
- As pre-ovulatory follicles are difficult to suppress, if cycle stage is not known, SEPARATE animal for 1 – 2 weeks (IM Vs SC)

The following material comes from the AZA Contraception Advisory Group

http://www.stlzoo.org/animals/scienceresearch/contraceptioncenter/contraceptionrecommendatio/contraceptionmethods.htm. (Accessed 11th May 2009). Note that implanon (above) has only recently become available in the USA, but has been widely used in Australasia and Europe for over 10 years.

THE USE OF ANY CONTRACEPTIVE IN NON-DOMESTIC ANIMALS IS CONSIDERED EXPERIMENTAL
(M=MALE-DIRECTED, F=FEMALE-DIRECTED METHOD)

Implant - MGA (melengestrol acetate)

Manufacturer - ZooPharm division of Wildlife Pharmaceuticals, Colorado, USA.
Product Information - MGA implants are the most frequently used and consequently the contraceptive method for which we have the most information in the Wildlife Contraception Centre database. Melengestrol acetate is a synthetic progestin. MGA implants contain 20% melengestrol acetate by weight in a silastic matrix. ***Because different species require different dosages, implants are not interchangeable. Please check with the WCC regarding implants that are not being utilized.*** Although duration of MGA implant efficacy may vary by individual and species, the continued recommendation is to replace them at 2-year intervals.
Storage – Implants should be stored at refrigeration temperatures (4°C).

Sterilization – MGA implants should be inserted using sterile surgical technique. In addition, it is recommended that implants be ***gas-sterilized*** with ethylene oxide followed by ***de-gassing*** at room temperature for a minimum of ***2 weeks*** prior to use. Because the implants are porous, they must be de-gassed longer than metal instruments. Inadequate de-gassing may result in residual gas that may evoke a tissue reaction. If ethylene oxide sterilization is not available, the implant may be rinsed with alcohol and dried with sterile gauze prior to placement. Sterilization with a cold-soak solution is not recommended, because the chemicals can be absorbed and/or MGA may be leached from the implant. Low temperature hydrogen peroxide gas sterilization (STERRAD) is replacing the more dangerous EtO process in most hospitals. (More information can be found at www.sterrad.com). Our lab test found no difference in MGA release rates after implant sterilization with the STERRAD system, but long-term efficacy of these implants has not yet been evaluated. Because heat may change the structure of the MGA, ***implants should not be autoclaved.***

Insertion - Implants should be inserted between the scapulae intra-muscularly if possible, but, if subcutaneous placement is necessary, place implant in a "tunnel" created by blunt dissection of fascia away from the incision. Migration may be controlled by suturing the implant in place at the time of insertion.

Implant loss can be reduced by properly sterilizing implants before insertion, using sterile insertion technique, and separating the animal from conspecifics during the period of healing. (NOTE: in some taxa such as the callitrichids and small prosimians, steel sutures have been successful in preventing over-grooming and implant removal by conspecifics, thereby avoiding the need to separate animals). ***The implant's presence and location should be confirmed whenever the animal is handled.***

Monitoring implant placement - Identification transponder microchips inserted in MGA implants can be used to confirm presence and location. Implants cannot be supplied with transponders already in place; however, chips can be inserted in implants that are longer than the chip. Using sterile procedure, puncture implant longitudinally with needle containing transponder chip (it comes sterile) and insert into implant as you would under the skin. Insert implant into animal using standard surgical technique as outlined above. Secondly, stainless steel suture or comparable material may be incorporated into the implant to make it visible on radiographs prior to sterilization.

Implant disposal – used implants received from ZooPharm or Ed Plotka should be disposed of in proper waste containers after use.

Latency to effectiveness - Although individuals vary, threshold levels of the hormone should be reached in the blood within 1 to 3 days following IM insertion and within 1 week after SQ insertion. However, pre-ovulatory follicles are difficult to suppress, so, if cycle stage is not known, extra time must be allowed. Therefore, ***separation or alternative contraception should be used for at least 1week (if IM) or 2 weeks (if SQ) following insertion.***

Oestrous cycles during treatment - MGA may affect contraception by blocking ovulation, causing thickening of cervical mucus, slowing ovum transport, and/or interfering with fertilization or implantation. However, follicle growth may continue and sometimes be accompanied by oestrogen production sufficient to cause oestrous behaviour. Ovulation may occur even though pregnancy does not ensue. Higher progestin doses may be preferred, so that oestrous behaviour is prevented, but may not be effective in completely suppressing follicle growth and some estradiol production.

Duration of efficacy and reversibility - Implants are considered effective for at least 2 years and possibly much longer, depending on species and individual differences, but in some cases have been found to be effective for as much as 5 years when left in place. This means that ***implants should be replaced every 2 years to insure contraception, but should be removed when pregnancy is desired***. For this reason too, old implants should be removed when a new one is placed to avoid administering a higher than intended dose. Once the implant is removed, the circulating MGA clears very rapidly, so that ovulation and conception may occur within days, although actual latency is usually longer and will depend on the individual.

Use during pregnancy - Synthetic progestins like MGA are not recommended in pregnant animals because of the possibility of prolonged gestation, stillbirth, abortion, etc. in some species, although the effect may depend on dose. Progestins in late pregnancy seem not to interfere with parturition in primates, but this may be a taxon-specific phenomenon.

Use during lactation - Progestins are sometimes prescribed for lactating women and are considered generally safe for nursing infants.

Use in pre-pubertals or juveniles – Future reproduction was not affected in calves of domestic cows on MGA-treated feed, but no studies of pre-pubertal treatment with MGA or other progestins have been conducted with other species, so possible long-term effects on fertility are not known.

Precautions – MGA can cause weight gain in all species. Possible deleterious effects on uterine and mammary tissues vary greatly by species; see cautions for each taxon.
Consideration for seasonal breeders - Treatment should begin at least one month before the anticipated onset of the breeding season. However, in canids, treatment should begin more than two months before the time of anticipated oestrus, because proestrus increases in estradiol can begin as much as two months before oestrus, and it is known that this endogenous estradiol can exacerbate deleterious effects of progestins on the uterus and mammary glands. This synergy of estradiol and progestins may also occur in other carnivores, such as mustelids and ursids.
Reporting Requirements - All institutions must submit a complete Contraception Centre Survey to the AZA Wildlife Contraception Centre. The product will no longer be sold to any institution that fails to submit the annual survey.
Request for purchase – MGA implants may be purchased by prescription through ZooPharm. All prescriptions should be written using their protocol and ***MUST include an authorization number*** designated by the AZA Wildlife Contraception Centre. MGA implants cost $30/gram (As of June 1st 2008) plus shipping and handling. To request authorization for ordering MGA implants, please complete the Implant Request Form and submit to:
Sally Boutelle, Program Coordinator
AZA Wildlife Contraception Centre
Saint Louis Zoo
1 Government Drive
St. Louis, MO 63110
314-646-4595; fax: 314-646-5534
Contraception@stlzoo.org

Injection - DEPO-PROVERA® (medroxyprogesterone acetate)
Manufacturer – Pfizer
Product information - With the second most numerous records in the Wildlife Contraception Centre database, Depo-Provera® has been used most often in reproductively seasonal species (e.g., prosimians, bears, pinnipeds), species in which anesthesia for implant insertion is problematic (e.g., giraffes, hippos), and as an immediately available interim contraceptive (e.g., if an implant is found missing or has not been ordered). Medroxyprogesterone acetate is a synthetic derivative of progesterone administered as an acetate salt with anti-estrogenic activity.
Dose - Dosage studies have not been conducted for most species. Recommended doses and injection intervals vary according to species and experience. Current reports have indicated that 2-5 mg/kg body weight every 2-3 months has been effective (the higher dose for smaller species and the lower dose for larger ones). However, New World monkeys require as much as 20mg/kg monthly. For especially large species for which body weights may not be available, such as hippos, see Taxon-Specific Recommendations.
Latency to effectiveness - IM injection is roughly equivalent to implant insertion, i.e., separation or alternative contraception should be used, conservatively, for 2 weeks, but at least for1 week.
Oestrous cycles during contraceptive treatment - Synthetic progestins may affect contraception by blocking ovulation, causing thickening of cervical mucus, slowing ovum transport, and/or interfering with fertilization or implantation. However, follicle growth may continue and sometimes be accompanied by

oestrogen production sufficient to cause oestrous behaviour. Ovulation may occur even though pregnancy does not ensue. Higher progestin doses may be preferred, so that oestrous behaviour is prevented, but may not be effective in completely suppressing follicle growth and all estradiol production.
Duration of efficacy and reversibility - Duration of efficacy, and thus latency to conception following last injection, can be extremely variable and has been seen to vary from 4 weeks to 2 years in some individuals. In general, the recommended dose (2.5-5 mg/kg BW) is effective for at least 2 months in most species. Hippos and giraffe have been treated at lower doses and appear to need re-treatment every 6 weeks. New World primates require higher doses at more frequent intervals.
Use during pregnancy - Progestins are not recommended in pregnant animals because of the possibility of prolonged gestation, stillbirth, abortion, etc. in some species, although the effect may depend on dose. Progestins in late pregnancy seem not to interfere with parturition in primates, but this is a taxon-specific phenomenon. Because of the variability in duration of efficacy for Depo-Provera, special caution should be used when treating females that might be pregnant.
Use during lactation - Progestins are sometimes prescribed for lactating women and are considered generally safe for nursing infants.
Use in pre-pubertals or juveniles - Future reproduction was not affected in calves of domestic cows on MGA-treated feed, but no studies of pre-pubertal treatment with MGA or other progestins have been conducted with other species, so possible long-term effects on fertility are not known.
Consideration for seasonal breeders - Treatment should begin at least one month before the anticipated onset of the breeding season. This does not include however canids or other carnivores due to the potential for progestin side effects addressed in the corresponding taxonomic sections below.
Precautions – Progestins likely cause weight gain in all species. Possible deleterious effects on uterine and mammary tissues vary greatly by species; see cautions for each taxon. In the human literature, Depo-Provera® has been linked to mood changes. Because it binds readily to androgen receptors and is anti-estrogenic, females may experience male-like qualities (increased aggression, development of male secondary sex characteristics, etc.)
Reporting requirements - All institutions using Depo-Provera® are asked to submit a complete Contraception Centre Survey to the AZA Wildlife Contraception Centre. It is essential that accurate records of doses and intervals be maintained and results reported to the Wildlife Contraception Centre Database to contribute to dosage development.
Please submit surveys to:
Sally Boutelle, Program Coordinator
AZA Wildlife Contraception Centre
Saint Louis Zoo
1 Government Drive
St. Louis, MO 63110
314-646-4595; fax: 314-646-5534
Contraception@stlzoo.org

The Pill
- Various progestin plus oestrogen analogues
- Follow human protocol – so must take each day

- Treatment can begin in any phase of the cycle, but may not be effective in the first month if treatments begins near the time of ovulation - Impractical in most situations

PROGESTIN-ONLY PILLS

- Ovrette® (norgestrel) pills – 0.075mg
- Jolivette® (norethindrone) pills – 0.35mg
- Micronor® (norethindrone) pills – 0.35mg
- Nora-Be® (norethindrone) pills – 0.35mg
- Nor-QD® (norethindrone) pills – 0.35mg
- CamilaTM (norethindrone) pills – 0.35mg
- ErrinTM (norethindrone) pills – 0.35mg

The limited data in the WCC database regarding the use of these orally active progestin-only contraceptives is primarily for great apes and a few Old World monkeys.
Manufacturer – See brand details and the list of manufacturers: BC Pills
Product information – A progestin-only oral contraceptive pill consists of synthetic progesterone, either norgestrel or norethindrone, without oestrogen.
Latency to effectiveness - As with implants and injections, separation or alternative contraception should be used for 1-2 weeks after initiation of treatment.
Oestrous cycles during contraceptive treatment - Synthetic progestins may effect contraception by blocking ovulation, causing thickening of cervical mucus, slowing ovum transport, and/or interfering with fertilization or implantation. However, follicle growth may continue and sometimes be accompanied by estradiol production sufficient to cause oestrous behaviour. Ovulation may occur even though pregnancy does not ensue. Higher progestin doses may be preferred, so that estrous behavior is prevented, but may not be effective in completely suppressing follicle growth and some estradiol production.
Duration of efficacy and reversibility - Duration of efficacy may not be much more than one day, so pills must be administered daily. Following cessation of treatment, rapid clearance can result in ovulation within a few days, but actual latency to conception will vary by individual.
Use during pregnancy - Progestins are not recommended in pregnant animals because of the possibility of prolonged gestation, stillbirth, abortion, etc. in some species, although the effect may depend on dose. Progestins in late pregnancy seem not to interfere with parturition in primates, but this is a taxon-specific phenomenon. **Use during lactation** - Progestins are sometimes prescribed for lactating women and are considered generally safe for nursing infants.
Use in pre-pubertals or juveniles - Future reproduction was not affected in calves of domestic cows on MGA-treated feed (another synthetic progestin), but no studies of pre-pubertal treatment with MGA or other progestins have been conducted with other species, so possible long-term effects on fertility are not known.
Consideration for seasonal breeders - Treatment should begin at least one month before the anticipated onset of the breeding season.
Precautions – Progestins likely cause weight gain in all species. Possible deleterious effects on uterine and mammary tissues vary greatly by species; see cautions for each taxon.

Reporting requirements - All institutions using Progestin-only pills are asked to submit a complete Contraception Center Survey to the AZA Wildlife Contraception Center. It is essential that accurate records of doses and intervals be maintained and results reported to the Wildlife Contraception Center Database to contribute to dosage development.
Please submit surveys to:
Sally Boutelle, Program Coordinator
AZA Wildlife Contraception Center
Saint Louis Zoo
1 Government Drive
St. Louis, MO 63110
314-646-4595; fax: 314-646-5534
Contraception@stlzoo.org

2. GNrH Impants

SUPRELORIN® (deslorelin) IMPLANTS
Manufacturer – Peptech Animal Health, Australia
Product information – Suprelorin® (deslorelin), a GnRH agonist, effects contraception by temporarily suppressing the reproductive endocrine system and preventing production of pituitary (FSH and LH) and gonadal hormones (estradiol and progesterone in females and testosterone in males). The observed effects are similar to those following ovariectomy or castration, but are reversed after the hormone content of the implant is depleted. As an agonist, deslorelin first stimulates the reproductive system, which can result in estrus and ovulation in females or temporary enhancement of testosterone and semen production in males. Then, down-regulation follows the initial period of stimulation. Although deslorelin can also be an effective contraceptive in males, we recommend its use primarily in females, since monitoring efficacy in females by suppression of estrous behavior or gonadal steroids in feces is more straightforward than ensuring continued absence of sperm in males, since most institutions cannot perform regular semen collections. It can, however, be used to ameliorate aggression in males but higher dosages are usually needed.
Deslorelin implants are available in two formulations: 4.7-mg for a minimum of 6-month, and 9.4-mg for a minimum of 12-month contraception. Deslorelin has been tested primarily in domestic dogs and cats, which makes it most suitable for carnivores, and it has successfully reduced aggression in male lion-tailed macaques. However, it appears not to be effective in male bovids or marsupials. It is currently in use in a number of species but the primary taxonomic group treated has been carnivores.
Storage and Expiration – Implants should be stored at refrigeration temperatures (4°C). Expiration date is stamped on individual implant packages. If implant expires prior to placement, contact Sally Boutelle (contraception@stlzoo.org) for the actual longevity of the implant.
Insertion – The implant comes pre-loaded in an insertion device. The recommended site of implant placement is SQ between the shoulder blades. The area should be clipped and cleaned using standard surgical prep techniques. A fold of skin should be lifted and held between the thumb and fingers as the obturator (sent with the implant) is inserted. To prevent breakage of the implant during insertion, the barrel of the obturator should be slowly withdrawn as the

implant is expelled. The implant should be held steady as the obturator is removed to insure release of the implant so that it remains in place under the skin.
Latency to effectiveness - Because the initial effect is to stimulate the reproductive system, it is important to either separate treated animals from opposite sex individuals during the period of enhanced fertility or use another form of contraception. Females treated with deslorelin should be considered fertile for 3 weeks following insertion. Males may remain fertile for 2 or more months, until residual sperm either degenerate or are passed (as following vasectomy). Lessening of aggression in some male primates treated with deslorelin or other GnRH agonists was not seen for 6-12 months, but the delay may have been due to an inadequate initial dose.
Suppression of initial estrus/ovulation – The estrus and ovulation that can occur within 2 weeks following implant insertion can be suppressed with supplemental progestin treatment for 15 days (7 days prior to and 8 days after implant insertion). Megestrol acetate tablets are the simplest form for short-term progestin administration, with the tablet offered as a treat to insure ingestion. Depo-Provera® should not be substituted for Megestrol acetate, because its initial high levels and sustained release can interfere with Suprelorin® efficacy. MGA implants can be left in place for 2-3 weeks following Suprelorin® implant insertion, but then should be removed to prevent interference with the down-regulation action. Leaving them in place longer may compromise Suprelorin® efficacy.
Estrous cycles during contraceptive treatment - Deslorelin first stimulates, then suppresses estrus in females. Species with induced ovulation (e.g., felids, some mustelids, bears) may ovulate and become pseudo-pregnant (also canids) when first treated. In males, initial stimulation may be accompanied by increased aggression or sexual interest. Estrous behavior or even copulation may occur during a transition phase near the end of the period of contraceptive efficacy.
Duration of efficacy and reversibility – A new 12-month formulation containing 9.4mg deslorelin should be effective for approximately twice as long as the smaller (4.7mg) implants that have been supplied in the past. However, the dose needed per-kg-body-weight with the new 9.4mg implants is about twice that of the existing 4.7mg implants. For animals effectively contracepted for 6 months with two 4.7mg implants, two 9.4mg implants will be necessary, but the period of efficacy will be double (12 months). For 6 months contraception, one 9.4mg implants will not substitute for two of the 4.7mg ones. These dose recommendations should only serve as general guidelines, because individual animals may respond differently. Stated durations of efficacy should be considered minimums. The smaller implants may actually be effective for more than 6 months, and the larger ones for more than 12 months, in some animals. Data from various species have shown, responses may vary widely between individuals, but that the response from one individual tends to be consistent and if an individual reverses earlier than expected it will consistently do so. If it is not possible to wait for signs of reversal to determine duration of efficacy for the animal, then for continuous contraception the small implants should be replaced at 5- to 6-month intervals and larger ones at 11- to12-month intervals.
Use during pregnancy – GnRH agonists should not be used during pregnancy, as they may cause abortion.
Use during lactation - No known contraindications once lactation has bee development.

Use in pre-pubertals or juveniles – Because deslorelin suppresses gonadal steroids, its use may delay epiphyseal closure of the long bones, resulting in taller individuals, similar to the effects of pre-pubertal spaying and neutering in domestic dogs and cats. GnRH agonist use in prepubertal domestic cats was followed by reproductive cycles after treatment ceased. However, species differences may occur.
Consideration for seasonal breeders – In females, GnRH agonists can induce estrus and ovulation even during the non-breeding season in some taxa. In males, GnRH agonists can transiently stimulate testosterone production even during the non-breeding season. Treatment should begin more than two months prior to the anticipated breeding season to prevent initiation of spermatogenesis, because it appears that suppression of sperm production is more easily accomplished before it has commenced and time must be allowed for passage of residual sperm, as following vasectomy.
Precautions - In general, the effects on weight should be similar to those from ovariectomy or castration. Preliminary data indicate that increased appetite will result in weight gain, especially in females, unless food is restricted. In males, muscle loss may result in overall weight loss if not replaced by fat. In sexually dimorphic species, males may become the size (weight) of females. Animals may loose secondary sex characteristics (e.g. lions may loose the mane while being treated with deslorelin).
Reporting requirements - All institutions using deslorelin must submit a complete Contraception Center Survey to the AZA Wildlife Contraception Center. ***The product will no longer be sold to any institution that fails to submit the annual survey.***
Request for purchase - Deslorelin implants are available to AZA accredited institutions as part of a research trial coordinated by the AZA Wildlife Contraception Center as part of an agreement with Peptech Animal Health, Australia . This product is not commercially available in the United States at this time. For those institutions outside the U.S. interested in deslorelin, contact Peptech Animal Health directly for information at www.peptech.com. For AZA accredited institutions in the U.S. please submit the Deslorelin Agreement Form to:
Sally Boutelle, Program Coordinator
AZA Wildlife Contraception Center
Saint Louis Zoo
1 Government Drive
St. Louis, MO 63110
314-646-4595; fax: 314-646-5534
Contraception@stlzoo.org

REVERSIBLE VASECTOMY
This technique has been used successfully in thousands of lab rodents and humans (90% success rate in more than 4,000 cases: Silber & Grotjan 2004), but has only been attempted in a few exotic species, so should be considered experimental. Reversals have been accomplished in bush dogs (DeMatteo et al. 2006) and most recently in a Przewalski's Horse. Initial vasectomies have been performed in chimpanzees but reversals not yet attempted. Thus, the procedure should not be used in males likely to be recommended for subsequent breeding until more experience is gained with a broader range of species.

To increase the chance of successful reversal, it is important that an "open-ended" vasectomy be performed, leaving the distal (testicular) end open to permit leakage, which allows a pressure-relieving granuloma to form, minimizing vas or epididymal damage (Silber 1977a). The proximal (abdominal) end can be cauterized, providing an effective seal which prevents spontaneous recanalization (Silber 1976, 1977a,b)

Reversal surgery is possible subsequent to other vasectomy procedures but requires a very difficult anastomosis of the vas to the epididymis to reverse. The "open-ended" vasectomy permits reversal via the much simpler vasovasostomy (Silber 1977a, 1978, Silber et al. 1977).

Open-Ended Vasectomy Procedure - The typical midline incision used in neutering results in difficulty freeing the proximal end of the vas deferens during the subsequent reversal procedure. Rather, the vas should be isolated from the cord via a small incision in the upper scrotum or at the external inguinal ring. Because the thickness of the scrotal skin may preclude the scrotal approach used for humans, a 1- to 1.5-cm incision should be made over the external inguinal ring. The vas deferens should be kept moist by pulsatile irrigation with heparinized saline (2500U heparin/500 ml NaCl) to avoid post-operative scarring. After the vas deferens is transected, the abdominal (proximal) cut end should be cauterized by inserting a needle electrode about 1 cm internally. If only the mucosa is cauterized, leaving the muscle is unharmed, a very tight seal will form to achieve blockage. The distal end should be left open for leakage to release pressure.

Vasovasostomy - Dr. Sherman Silber, the physician who pioneered the technique, has offered his services to the zoo community to perform reversals. If there is sufficient interest among zoo veterinarians, we can organize training session in the technique. Otherwise, Dr. Silber should be contacted to perform the surgery. In general, the procedure entails making an incision over the upper scrotum and external ring similar to the original incision for the vasectomy, exposing the vas deferens longitudinally by blunt dissection, facilitated by placing a small Penrose drain underneath the vas. The distal and proximal ends of the vasa are held with vasovasostomy clamps and the scarred ends of both sides are resected. Translucent fluid is aspirated from the distal cut end with 22 g medicut and 1-cc syringe to check for the presence of sperm. Absence of sperm in the fluid may indicate an epididymal blockage which makes successful vasovasostomy unlikely. However, if the original vasectomy was open-ended, this complication is very unlikely. The vasovasostomy is performed using 9-0 nylon interrupted mucosal sutures and 8-0 nylon interrupted muscularis sutures.

Latency to Effectiveness - Latency to disappearance of sperm following vasectomy will depend on the species and individual, perhaps as long as 2 months, until residual sperm either degenerate or are passed.

Precautions - Vasectomy is not recommended for species with induced ovulation because mating will result in female pseudopregnancies with prolonged periods of progesterone elevation, which can cause pathology of uterine and mammary tissue. Endogenous progesterone and progestin contraceptives cause similar disease.

Contact for More Information - Dr. Sherman Silber (DrSherm@aol.com)

References:

DeMatteo, K.D., Silber, S., Porton, I., Lenahan, K., Junge, R., Asa, C.S. 2006. Preliminary tests of a new reversible male contraceptive in bush dogs

(*Speothos venaticus*): Open-ended vasectomy and microscopic reversal. J. Zoo Wildl. Med.

Silber S.J. 1976. Microscopic technique for reversal of vasectomy. Surg. Gynecol. Obstet. 143: 630.

Silber S.J. 1977a. Sperm granuloma and reversibility of vasectomy. Lancet 2:588-589.

Silber S.J. 1977b. Perfect anatomical reconstruction of vas deferens with a new microscopic surgical technique. Fertil. Steril. 28:72.

Silber S.J. 1978. Vasectomy and vasectomy reversal. Fertil. Steril. 29:125-140.

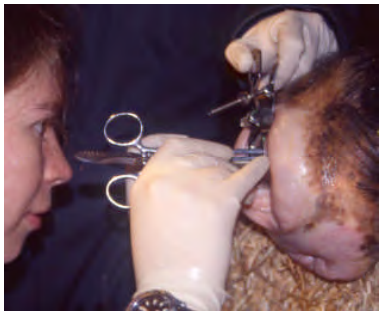
Silber S.J., Galle, J., and Friend, D. 1977. Microscopic vasovasostomy and spermatogeneis. J. Urol 117:299.

Silber S.J., and Grotjan, H.E. 2004. Microscopic vasectomy reversal 30 years later: a summary of 4010 cases by the same surgeon. J. Androl. 25:845-859.

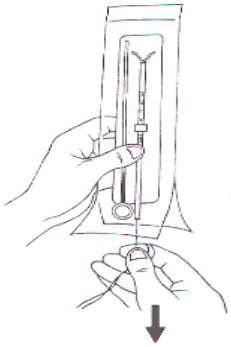
IUD's

- Intra Uterine Devices.
- Very cheap – 1000CFA each
- Some skill required to place
- Poor success rate in juveniles or individuals that haven't given birth (nullviporous) – will fall out
- BUT – don't alter social hierarchy or oestrus cycles
- Acts as a physical barrier

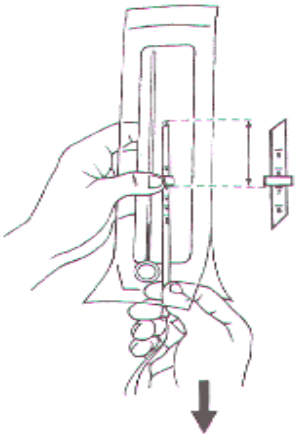
IUD Placement 1
Measure uterine depth



IUD Placement 2

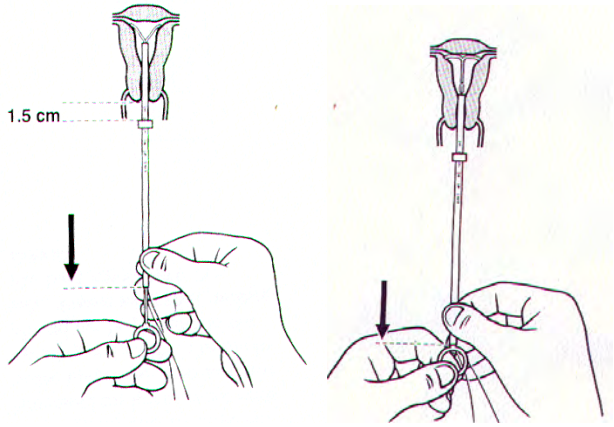


Withdraw device into sleeve with string.



Set depth marker from previous uterine measurements

IUD Placement 3



Which contraception method to use?

Considerations	**Contraception type**			
Adv Disad	IUD	Pill	Implant	LA injections
Reversible	+	+	+	+/-
Behavioural Oestrus	+	+	+/-	-
Safe for recipient/operator	+	-	+	+
Effective	+/-	+	+	+
Target specific individuals	+	+	+	+
Cost /5yr (£)	10	40	150	90

CHIMPANZEE AND GORILLA

Recommendations (Does not include Implanon, as American recommendations, however, the authors would put implanon as currently the most reliable chemical contraceptive for Apes.

(1) **MGA implant (F)**

(2) **Birth Control Pills** (for females that reliably take medicated treats) **(F)**

(3) **Depo-Provera (F)**

(4) **GnRH Agonists** - Gonadotropin Releasing Hormone Agonists are considered the safest reversible contraceptives, but dosages and duration of efficacy are not well established for all species.

- Suprelorin® (deslorelin) Implants **(F or M)**
- Lupron® Depot Injection **(F or M)**

(e) Cautions

(1) Chimpanzee sexual swelling: available data show that **females exhibit partial to normal swellings on birth-control pills, and partial to no swellings on MGA**, with differences likely due to relative doses or pill regimen that includes a placebo week.
(2) Combination birth control pills are NOT recommended during the first year of lactation because the Estrogen can suppress milk production.

Research and Monitoring

(1) Surveillance for deleterious effects Contact Dr Sue Walker s.walker@chesterzoo.org (Europe) or Sally Boutelle contraception@stlzoo.org (USA)

OLD WORLD MONKEYS
Recommendations
(1) **GnRH Agonists** - Gonadotropin Releasing Hormone Agonists are considered the safest reversible contraceptives, but dosages and duration of efficacy are not well established for all species; males may require higher doses. Side effects are generally similar to those associated with gonadectomy, especially the potential for weight gain unless diet is controlled.

- Suprelorin® (deslorelin) Implants **(F or M)**
- Lupron® Depot Injection **(F or M)**

(2) **MGA implant (F)**
(3) **Depo-Provera® injection** (2.5-5 mg/kg body wt. at 45-90 day intervals throughout breeding season) **(F)**.
Cautions

(1) No deleterious effects noted, although caution is suggested with progestin use

Research and Monitoring
(1) Chart sexual swelling in species for which it applies

(f) **(2) Surveillance for deleterious effects - Contact Dr Sue Walker s.walker@chesterzoo.org (Europe) or Sally Boutelle contraception@stlzoo.org (USA)**

3.17 DIAGNOSTIC SAMPLING PROCEDURES

S.Unwin, F.Leendertz, W.Boardman, M.Ancrenaz and W.Bailey

Researchers are directed to Cheeseborough (2005) as an excellent resource on field laboratory techniques. Leendertz et al (2006) also provides a good overview of techniques for biological sampling from primates in the field for disease investigations.

Sample collection and media that can be stored at room temperature.
A range of media exist that store biological samples without the need for special techniques. There use is dependant on what the sample is required for and include:

10% buffered formaldehyde Use – cellular structure (Histology, histopathology)
Tissue samples of maximum size 0.5cm x 0.5cm preserved in 10% Formalin. Samples from different organs can be pooled in one pot of formalin. Make sure you use enough formalin (at least 1Volume sample: 10 Volume Formalin). Samples preserved in Formalin are non infectious. Faeces may also be preserved in 10% formalin for parasitology, but in a separate container to the tissue samples.

RNA-later Use - PCR, Immunology (DNA/RNA and antibodies)
Tissue samples of maximum size 0.5cm x 0.5cm preserved in RNA-later. Samples from different organs should be placed in different tubes and labelled accordingly. Samples in RNA-later can be stored at room temperature up to 2 weeks. Beyond this they should be transferred into a fridge or freezer. Thawing of samples does not affect the sample quality, and shipment can be done at room temperature.

Dried blood (and little pieces of tissue) **Use - PCR and Immunology** (DNA/RNA and Antibodies)
Blood is preserved on special filter paper which is placed in a 50 ml tube over silica gel (for drying). Put 2-3 drops of blood on the filter paper, so it is soaked with blood and close the tube well. If possible store in a fridge, shipment is possible at room temperature. In case RNA-later is not available you may also preserve little stripes of tissue samples on this filter paper. Stripes should be as thin as possible.

10% Glycerol Use - Culturing of bacteria
Put little pieces of tissue, blood or faeces in 10% glycerol. Pieces should be very small, maximum 0.3 X 0.3 cm. Use different tubes and label accordingly. Samples can be stored for about 2 weeks at room temperature, later they should be stored frozen. Sample shipment is possible at room temperature.

Many other biological samples require specific storage conditions (refrigeration or freezing) and rapid processing following collection.

However, relatively inexpensive field-compatible portable fridges, freezers and liquid-nitrogen containers are available, and several biological parameters can be screened easily *in situ* with diagnostic field kits, meaning that samples do not necessarily have to be shipped to a distant laboratory.

All samples should be carefully labelled (using a solvent-proof pen) with the date, location, species, individual identification, and the type and number of the sample taken.

FAECAL SAMPLES

Faecal samples are a 'goldmine' for biologists owing to the range of information they can provide concerning intestinal parasites, feeding ecology, seed dispersal, DNA, and endocrinology (Cha Faeces can be collected directly from the rectum when a primate is handled, or non-invasively during fieldwork. They are stored easily in the field and most analyses can be performed long after sample collection. Faeces should never be handled without gloves because of the risk of zoonosis transmission.
When collecting faeces, note the colour, odour, size and consistency at the time of collection, along with major macroscopic elements, including the presence or absence of worms. Worms (if any) should be preserved in a 10% formalin solution for identification (as described above).

Faecal Parasite investigation – Formol-ether concentration technique
This method is simple and accurate for investigating all helminth and protozoal parasites. It is a good screening test. Other procedures may be required for specific parasites (see Cheeseborough 2005 for more information)
Equipment required (Figure 1):

- Fresh faecal sample
- 10% Formol Saline
- 15ml flat bottomed tube and lid*
- 15ml centrifuge tube*
- 1g plastic spoon*
- FPC strainer*
- Trixon X-100*
- Cotton tipped applicator *
- Wooden stick
- Plastic Pasteur pipette
- Ether or ethyl acetate
- Gloves
- Microscope slides and coverslips
- Centrifuge (hand powered suitable if no access to electricity) and Microscope

(* = Commercially available Evergreen FPC kit (Figure 1)

Figure 1. Components of the Evergreen FPC kit (W.Bailey)

Methodology:

- Wear gloves
- Add 10ml of Formal Saline 10% to the flat -bottomed tube in the kit
- Add 1x level spoon of fresh faeces
- Mix thoroughly with the wooden stick until there are no visible lumps of faecal material (Figure 2). If the sample is very hard, leave to stand for a while.
- Place white lid and shake sample vigorously

The sample can know be stored if necessary, for analysis if there is nowhere in the field to run this.

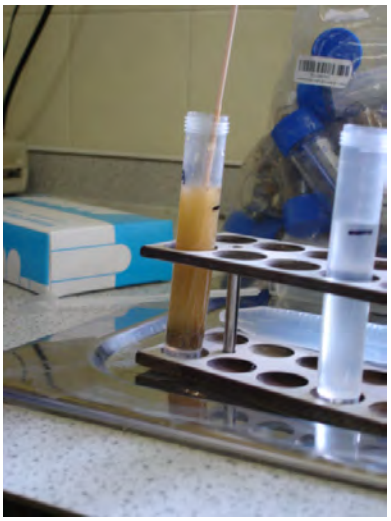


Figure 2 Mixed faecal sample in 10mL formol saline in the flat bottomed 15mL tube (W.Bailey)

- Ensure the FPC strainer is securely attached to the 15ml centrifuge tube. The strainer should be central on the vent-straw (Figure 3)
- Remove the white lid and attach the FPC strainer tightly to flat-bottomed tube. Shake vigorously.

Figure 3 Conical tube that comes with the Evergreen Kit, with the attached strainer and straw. (W.Bailey)

- Invert the tube, pointing the conical end of the centrifuge tube downward, and shake the specimen through the strainer
- If necessary tap the flat-bottomed tube to encourage the sample through the strainer
- Unscrew the FPC strainer and flat-bottomed tube
- Add approximately 1mL of ether or ethyl acetate and 2 drops of Triton X-100 to sample in centrifuge tube.
- Replace white lid and shake vigorously

If no centrifuge is available this sample can be left to settle for a minimum of 1 hour. Electric centrifuge- 1-2 minutes at around 1,500-3,000rpm, hand centrifuge (figure 4) for 2 minutes at about 90 rpm!

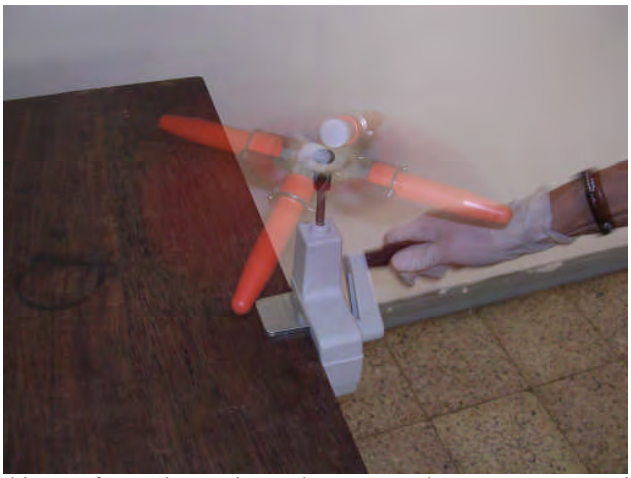


Figure 4. Manual Field Centrifuge. This can be used an areas without power, to spin down blood, urine, as well as faecal samples (W.bailey)

- Dispose of supernatant from centrifuge tube
- Remove any debris from tube using the cotton-tipped applicator
- Add a few drops of Formal Saline 10%, or physiological saline, to sediment and mix thoroughly
- Add 1 drop of sediment/formal saline mix to microscope slide.
- Place coverslip and examine under microscope (Figure 5). Scan slide at x10, then use x40 for further investigation/ confirmation of ova/ cysts or larvae.

Figure 5. Binocular microscope ideal for field use. (W.Bailey)

BACTERIOLOGY SPECIMEN COLLECTION GUIDELINES

Abscess: specimens should be collected by aspiration; pus from recently formed abscesses will yield the best cultural results. Remove surface exudates by wiping with 70% alcohol or sterile saline;
Open abscess: if possible aspirate material; if not pass a swab deep into the lesion and sample the tissue "fresh border";
Closed abscess: Aspirate material with needle and syringe; aseptically transfer ***all*** material into anaerobic transport device.
Pustules or vesicles: disinfect the surface with 70% alcohol, allow to dry and aspirate material with a sterile syringe and fine needle. If there is only a very small amount of aspirated material it can be left in the syringe, the air expelled and the needle covered.
Cellulitis:
1. Cleanse site by wiping with sterile saline or 70% alcohol;
2. Aspirate the area of maximum inflammation with a needle and syringe. Irrigation with a small amount of saline may be necessary.
3. Aspirate saline into syringe and expel aseptically into sterile tube or can be left in the syringe with needle removed and a sterile stopper placed over the needle place.

Exudates from draining sites, peritoneal and pleural effusions, pyometra, osteomylitis, abscess material, may contain **anaerobic bacteria** and need to be cultured immediately. Surface disinfection followed by aspiration or biopsy are the most appropriate for anaerobes recovery;
If the material is contained in a syringe, the air should be expelled and the needle removed. *Please do not refrigerate samples suspected of containing anaerobes as same species do not tolerate reduced temperatures;*
Wounds-can be sampled by dissecting a small portion of infected tissue. In the case of ***deep lesions*** that communicate with the surface, surgical debridement and sampling are recommended. If surgery is not performed, effort needs be made ***to aspirate*** a "pocket" of infected material that is not open to the surface;

Swabs-are most useful for obtaining specimens from**: ears, conjunctiva, deep within draining tracts or wounds, soft tissue infections or the reproductive tract specimens**. When swabs are used for collection, two swabs

should be used, one for culture and one for preparation of a smear; place the swab for culture in transport medium.

Outer ear:

1. Use moistened swab to remove any debris or crust from the ear canal;

2. Obtain sample by firmly rotating 2 swabs in the outer canal (one for direct smear and one for culture);

Eye (conjunctiva):

1. Sample both eyes with separate swabs (premoistened with sterile saline) by rolling them over each conjunctiva;
2. Collect a different swab for direct smear;

Upper respiratory tract:

Oral: 1. Remove oral secretions and debris from the surface of the lesions with a swab**;**

2. **Discard this swab.**

3. With a second swab, vigorously sample the lesion, avoiding any area of normal tissue;

Throat or pharynx: 1 Depress tongue with a tongue depressor;

2. Sample the posterior pharynx, tonsils and inflated areas with a sterile swab.

Lower respiratory tract: Transtracheal wash, lung aspirate or biopsy.

Faeces: Faecal specimen should be obtained direct from the rectum. "Ground droppings" should be avoided.

Routine culture: 1. Pass specimen directly into a sterile container.

2. Transport to the lab within 1 h of collection or transfer to Carry-Blair medium.

Rectal swab: 1. Insert a swab approx 1 cm beyond the anal sphincter;

2. Gently rotate the swab to sample the anal crypts.

3. Faeces should be visible on the swab for detection of diarrheal pathogens;

Blood: 3 or 4 blood samples should be collected. Disinfect culture bottle; apply 70% alcohol or phenolic disinfectant to rubber stopper and wait 1 min.; palpate vein before venipuncture.

Disinfection of venipuncture site:

1. Cleanse site with 70% alcohol;
2. Swab concentrically, starting at the centre with tincture of iodine;
3. Allow disinfectant to dry;
4. Do not palpate vein at this point without sterile glove;
5. Collect blood;

Fluids: abdominal, ascites, joint, synovial, bile, pericardial, peritoneal, pleural; paracentesis, thoracentesis;

1. Disinfect overlying skin with iodine preparation;
2. Obtain samples via percutaneous needle or surgery;
3. Always submit as much fluid as possible;
4. **Never submit a swab dipped into fluid.**

Catheter: 1. Cleanse the skin around the catheter site with alcohol or iodine;

2. Aseptically remove catheter and clip 5cm of distal tip directly into a sterile tube

3. Transport immediately to the lab to prevent drying.

Urine –should be collected aseptically by midstream, catheter or cystocentesis.
Transport immediately to the lab; if not possible refrigerate immediately. Do not leave at room temperature for longer than 1 hour.

EXAMINATION OF SKIN SCRAPINGS/HAIR BRUSHINGS FOR MITES & LICE

Equipment
- Sample (skin or hair)
- 5% KOH (made up from tablets with distilled water)
- Large beaker
- Bunsen burner
- Centrifuge
- Pipette
- Slide and coverslip
- Saturated salt solution
- Wire loop
- Microscope

Method
- Mix sample with 5% KOH at a ratio of 1:10 per volume
- Heat in the beaker over the Bunsen
- When hair/skin is dissolved allow to cool
- Transfer to tubes and centrifuge for 10 minutes
- Discard most of the supernatant and resuspend the sediment
- Top up with H_2O and mix
- Centrifuge again - let tubes stand for 5 minutes
- Parasites will float in the salt solution
- Remove top of supernatant with wire loop and put on slide and examine under microscope

BLOOD SAMPLING

Figure 6 shows femoral blood collection under field conditions, in a juvenile chimpanzee.

Figure 6. Femoral blood collection from a juvenile chimpanzee under field conditions (S Unwin)

Whole blood

For live individuals, whole blood is collected after venipuncture. Collection is made from the femoral vein for the smallest species, or when large quantities of blood are required for other species. The femoral vein is located in the femoral triangle, immediately adjacent to the femoral artery. When the primate is lying on its back, its legs in slight extension, the pulsation of the artery can be felt with the index finger, establishing the location of the vein. Prior to venipuncture, the sampling site should be disinfected with iodine solution. The smallest species (prosimians, marmosets and tamarins) are sampled with a 0.4 mm gauge needle and a 1 ml syringe; squirrel monkeys (*Saimiri* spp.) are sampled with a 0.9 mm needle and a 1 or 2.5ml syringe or a small vacuum tube, and larger species with a 1.2--2 mm needle and a 2.5--10 ml vacuum tube. Apply strong pressure to the vein for at least one minute following venipuncture to prevent haematoma formation, especially when the femoral artery has been inadvertently punctured. Other possible sites are the jugular vein (especially for the smallest species) or the tibial vein (prosimians, guenons or larger species). The tibial vein lies just under the skin on the caudal surface of the gastrocnemius muscle, and is easy to locate after compression of the upper thigh. However, this vein is rather small and collapses easily when large quantities of blood are collected. This is the site of choice for intravenous injections for all species of NHPs. The cephalic vein, or its branches, can be attempted in apes. In recently dead primates, blood can be obtained easily from the heart.

As a general rule, to prevent risk of hypotension and associated heart failures, blood sampling should never exceed 1 ml per 100 g body weight per month.

Haematological parameters

To prevent clotting, whole blood is placed in a collection tube containing sodium heparin or EDTA (ethylenediaminetetra-acetic acid, disodium salt) immediately following venipuncture. Red and white blood cell counts (RBCC and WBCC, respectively) are determined with a Coulter Counter or a Malassez' Cell slide after staining. Differential WBCC are made by examining 100 leucocytes in smears stained using the Wright--Giemsa method (Diff Quik™). These results provide valuable information on the medical status of the individual (presence of anaemia, infection, parasitism, neoplasm and other haematological abnormalities). Whole blood also allows the determination of other parameters (e.g. mean corpuscle volume, mean corpuscle haemoglobin) that explore haematological function more precisely. Haematocrit or packed cell volume (PCV) gives information on the general hydration level, and is determined by centrifuging blood-filled capillary tubes in a portable microcentrifuge. These analyses are carried out from a few hours to a few days (two to five days) following venipuncture (if the samples are stored in a fridge at 4--8 ◦C). To prevent haemolysis of blood cells, tubes must not be frozen or shaken.
The physiological range for biological parameters in captive NHPs can be found in the literature or the ISIS/MedArks system (available from all zoos and most captive facilities holding NHPs). See Section 3.19.

Bacteria and virus isolation

The isolation of bacteria and viruses from whole blood requires strictly aseptic conditions and sterile sampling equipment to prevent any bacterial contamination. Blood is collected in a sterile container with transport medium (sterile buffered glycerine or other buffered preparations used in cell cultures, with added bovine serum albumin (BSA) and antibiotics in the case of virus isolation). Samples are placed on wet ice (but not frozen) and sent directly to the laboratory since most viruses and bacteria remain viable for two to four days if kept cold. If a delay is expected, freeze the blood in a mixture of CO2 and alcohol, or in liquid nitrogen, and store at −70 ◦C or below.

Blood smears

Blood smears are easy to make, but hard to make well, and yield information concerning haematology, blood parasites and bacterial infection. Peripheral or capillary blood is collected from the tip of a finger or an ear, or can be taken as part of a larger blood sample. Disinfect the sampling site, slightly puncture the skin with a small needle, and collect a drop of blood. To make a thin blood smear place a single drop of whole or peripheral blood near one end of a horizontal microscope slide. Bring the end of a second slide up to the drop at 45*o* until the drop disperses along its edge. Then push the second slide quickly and evenly towards the opposite end of the first slide. The smear should dry immediately (if it doesn't it is too thick). A capillary tube can be used to provide a precise, small volume of blood for the smear. To make a thick smear, place a drop of blood on a slide, and spread in a small circle with the tip of a needle or the corner of a second slide for at least 30 seconds. After a few minutes at ambient temperature the smears are dry and

can then be transported to a laboratory or analysed in the field after staining or fixation in 90% methanol. Take a minimum of three to five blood smears per individual.

Blood drops

Small amounts of blood can be collected onto specially designed filter paper to test for antibodies against specific pathogens, or for hormonal analysis. When the blood spots are dry, place the filter papers in individual plastic bags or in normal envelopes with a desiccant and store in a dry place.

Plasma

Plasma is obtained by centrifugation of unclotted whole blood, and separation of the yellow/tan liquid material (plasma) from the clotted component (cell membranes and other blood composites). This is divided into aliquots in small plastic vials (0.5--2.5 ml), and stored frozen (−20 ◦C or below). Manual centrifuges (hand operated, figure 4) are available for field use where electricity supply is an issue.

Serum

To collect serum, place whole blood in special serum separator tubes or dry sterile blood collection tubes immediately after venipuncture. Leave the tubes undisturbed for at least one hour at ambient temperature to encourage clot formation and then centrifuge at 2000 *g* for 15--20 minutes. When a centrifuge is not available, serum can still be obtained by letting the clot or blood cells settle for few hours, then aspirating the liquid phase (serum). In order to maximise the quantity of serum, allow the blood to clot with the collection tube inverted (rubber stopper down). After a few hours, turn the tube stopper up and carefully remove the stopper with the blood clot attached, leaving the serum in the tube (Munson, 2000). Divide the serum into several aliquots in small vials and refrigerate or freeze.

SERUM/PLASMA STAINS

Giemsa stain

- Place blood film in absolute methyl alcohol for 3-5 minutes and air dry
- Fill the coplin jar with Giemsa stain prepared as follows: One volume of Giemsa stock solution to 9-25 volumes of distilled Place the blood film into the stain for 30-45 minutes.
- Wash the film with neutral distilled water for 30 minutes and drain dry.

Wrights Giemsa

- Dip slide in stain for 10-15 seconds
- Dip slide in distilled water for 15-30 seconds
- Rinse for a few seconds in distilled water and dry.

Diff Quick (LEUKOSTAT™)

- Dip blood smear in fixative solution 5 times, one second each time. Drain excess fixative
- Dip into solution 1 five times, one second each time. Drain excess solution.
- Dip into solution 11 five times, one second each. Drain excess solution
- Allow to dry and examine

Note: Keep coplin jars tightly closed when not in use. If you desire a paler or more intense stain, deep less or more in solutions 1 and 11.

New Methylene blue

This is used for rapid examination of unfixed blood films, bone marrow, exudates and impressions of biopsied materials, blood parasites, urine sediments.

- Prepare blood or other film on a slide or cover slip
- Place a drop of stain on a clean cover slip if film is on a slide or on a clean slide if film is on a cover slip.
- Avoid bubbles as stain spreads between glass surfaces.
- Examine wet mount with low power, high dry or oil immersion objectives.
- This film is not permanent but can be preserved for several hours by ringing edges of the cover glass with immersion oil.

HAEMATOLOGY AND BLOOD GLUCOSE - refer to section 3.19

URINALYSIS – refer to section 3.19

CYTOLOGY

Fine Needle Aspiration

Equipment

- Syringe containing sample in the needle.
- 2 clean slides.

Staining material

Method

- Detach the needle from the syringe.
- Fill the syringe with air and reattach the needle.
- Force the contents of the needle onto the slide.
- Don't force too hard or cellular contents will be damaged, don't force too soft of the contents will be left in the needle.
- Using the other slide, prepare a squash preparation in a similar manner as the slide and coverslip technique for avian blood smears except use slightly more downwards pressure.
- Stain using the Diff-Quik technique
- Examine prepared smear under microscope, both slides can be used for examination.

Body Effusions (Abdominocentesis, Thoracocentesis or Cystocentesis)

Equipment

- Syringe containing fluid.
- 2 clean slides.
- Sterile container
- EDTA tubes
- Centrifuge.
- Diff-Quik stain

Method

- Place a portion of the sample into a sterile container for bacteriology.
- Place the rest into a test tube (tubes) containing EDTA.
- Centrifuge these tubes for 15 minutes; ensure that the centrifuge is balanced.
- Using a pipette remove most of the supernatant, leaving a small amount with which to resuspend the sediment.
- The supernatant may be used to determine the specific gravity of the fluid using a refractometer. This may help determine protein concentration in the sample but is unreliable.
- Resuspend the sediment and using a pipette place a drop on a slide.
- Smear the sample using the wedge technique as for blood
- Stain the smear using Diff-Quik.

Tracheal Wash

Method

- At least 5ml of fluid should have been collected.
- Save some in a sterile container for bacteriology.
- A number of slides should be prepared by doing squash preps in a similar manner to the slide and coverslip technique used for avian blood smears
- Stain the smears using Diff-Quik

Impression Smear

Equipment

- Excised mass
- Slides
- Paper towel
- Forceps
- Diff-Quik stain

Method

- Cut the mass so as to give a fresh cut surface of no more than $1cm^2$
- Using the forceps, blot the cut surface against the paper towel until no more blood is removed on the towel until no more blood is removed on the towel. If the surface is too wet due to capillary ooze, the quality of the slide will be affected.
- Using the forceps, touch the cut surface to a slide (only touch, don't press). Take care not to smear the imprint.
- Make several imprints per slide and make several slides.
- Allow to dry
- Stain using Diff-Quik

MICROBIOLOGICAL and CYTOLOGICAL STAINING

Gram Stain

Equipment

- Grams crystal violet (0.5%)
- Grams iodine
- Decolouriser (acetone)
- Safranin (0.5%)

Method

- Make smear and air dry and heat fix
- Place slide on staining rack
- Flood with crystal violet and let stand for 30 seconds
- Rinse briefly with tap water
- Flood with grams iodine and let stand for 30 seconds
- Rinse briefly with tap water
- Using forceps hold the slide at a angle and drip decolouriser down the slide until it runs clear
- Rinse with tap water
- Flood with safranin counterstain and let stand for 30 seconds
- Rinse with tap water
- Allow to air dry
- Fully label slide
- Examine under microscope

Modified Ziehl-Neilsen Stain

For the identification of acid fast organisms e.g. Mycobacteria.

Equipment

- Air dried and heat fixed smear
- Dilute carbol fuchsin (stock solution diluted 1:10)
- Decolouriser (0.5% acetone)
- Methylene blue (1%)

Method

- Place slide on staining rack
- Flood with dilute Carbol Fuchsin and leave for 10 minutes
- Rinse with tap water
- Using forceps hold the slide at a angle and drip on decolouriser until it runs clear
- Rinse with tap water
- Flood the slide with Methylene blue for 20 seconds
- Allow to air dry
- Fully label slide
- Examine under microscope

Lactophenol Cotton Blue

For the staining of fungal hyphae

Equipment

- Air dried smear
- Lactophenol cotton blue

Method

Simply put a drop of lactophenol blue onto the smear; leave for a few minutes, wash it off and examine.

Table .1. Methods for taking biological samples, analysis, storage and associated costs (Cheeseborough 2005, Leendertz et al 2006,)

Sample	**Analysis**	**Examples for specific use**	**Equipment required**	**Storage**[5]	**Time**	***In situ* analysis**	**Costs**[6] Sampling	Storage	Analysis
Whole blood	Haematology	Red blood cell count, white blood cell count	EDTA[7] or heparinised tube, microscope, slides, colouration kit	Fridge	Max. 4 days	Possible	$	$$	$$
		Haematocrit (Pack Cell Volume)	Capillary tubes, microcentrifuge	Fridge	Max. 4 days	Easy	$	-	$
		Thin blood smear: differential white blood cell count	Microscope slides	Room Temp	Max. 4 days	Easy	$	-	$
	Pathogens	Thin blood smear: bacteria	Slide, colouration kit[8], microscope	Room Temp	Months	Easy	$	$	$
		Thick blood smear: haemoparasites	Slide, colouration kit, microscope	Room Temp	Years	Easy	$	$	$
		Virus or bacteria isolation/ PCR	Sterile equipment. Sterile transport media	Wet Ice Frozen	Few Days Months	Possible Possible	$$ $$	$$ $$$	$$ $$
	Toxicology	Pesticides	Dry (serum) tube (e.g. ependorf)	Frozen	Months	Unlikely	$	$$$	$$$
	Immunology	Antibodies	Whatman-type filter paper	Cool place	Months	Possible	$	$	$$
	Genetics	DNA	EDTA or heparinised tube, Centrifuge, Buffy coat on Whatman type filter paper[9], Dry (serum) tube (e.g. ependorf).	Filter paper (room temp) tube (frozen)	Months	Yes	$	$$	$$$
Plasma	Chemistry	Vitamins, minerals and metals	EDTA or heparinised tube, centrifuge (to extract plasma)	Frozen	Months	Unlikely	$	$$$	$$$
Serum	Biochemistry	Enzymes, electrolytes	Dry (serum) tube, centrifuge (optional – can tilt the tube	Fridge	Few days	Possible	$	-	$$

[5] Fridge, +4°C; frozen, -20°C or lower; room temperature +15-25°C
[6] Figures are indicative of the relative costs; from very low cost ($) to expensive ($$$)
[7] EDTA, ethylenediaminetetra-acetic acid, disodium salt
[8] Type of kit will depend on what investigating – for example – Grams stain for bacteria, Fields stain for various parasites, Diff Quik™ for blood smears.
[9] **Dried blood** (and small pieces of tissue) for PCR and immunology (DNA/RNA and antibodies). Blood can be preserved on special filter paper which is placed in a 50 ml tube over silica gel (for drying). Put 2-3 drops of blood on the filter paper, so it is soaked with blood and close the tube well. If possible store in a fridge, but shipment is possible at room temperature. If RNA-later is not available you can also preserve little strips of tissue samples on this filter paper. Strips should be as thin as possible.

			and wait for the clot to form).						
	Endocrinology	Hormones	Dry (serum) tube, centrifuge (optional – can tilt the tube and wait for the clot to form).	Frozen	Months	Yes	$	$$$	$$
	Immunology	Globulins, antibodies	Dry (serum) tube, centrifuge (optional – can tilt the tube and wait for the clot to form).	Fridge Frozen	Few days Months	Possible	$	$$$	$$$
	Toxicology	Pesticides	Dry (serum) tube, centrifuge (optional – can tilt the tube and wait for the clot to form).	Fridge Frozen	Few days Months	Unlikely	$	$	$$$
Faeces	Parasitology	Intestinal Parasites	Formol-ether centrifugation kit, slides, microscope, 10% formalin or 70% alcohol, kit for nematode culture	Room Temp	Months	Easy	$	$	$
		Protozoa	Slide, colouration kit, microscope, 10% formalin	Room Temp	Months to years	Easy	$	$	$
	Others	Good alternative for 'non invasive studies' of diseases. Genetics, viral detection, bacteriology, toxicology, immunology, endocrinology etc.	RNA Later, frozen, dried, 10% glycerine (depending on what you are trying to detect. See below for details).	Room Temp	Few days to years	Possible	$$	$$	$$
Urine	Endocrinology	Hormones	Dry collection tube	Frozen	Months	Possible	$	$$$	$$
	Urinalysis	Renal function	Dry collection tube, centrifuge, colouration kit, dipstix™	Room Temp	Day	Easy	$	$	$
	Pathogens	Virus, bacteria or	Sterile collection tube	Fridge	Few days	Possible	$	$$	$$$

		parasite isolation							
Tissues	Pathogens,	Virus or bacteria isolation	Swabs (e.g. rectal, oropharyngeal lesion).	Room Temp or Frozen	Years	Possible	$$	$ - $$$	$$
		Bacterial identification/ PCR	Swabs come with a variety of transport media (often	Room Temp	Years	Possible	$$	$	$
		Viral identification/ PCR	bacterial or viral specific)	Room Temp	Years	Possible	$$	$	$$
	Cytology	Alterations in cellular structure, presence of pathogens	that prolong the life of the sample. Dry swabs can be used for PCR. RNA Later, frozen and dried. Antibody detection (ELISA etc)	Room Temp	Years	Possible	$	$	$
			10% glycerine (or similar) [10]						
	Histology	Data on changes in tissues/ cells and pathogens	10% formalin[11]	Room temp (DO NOT FREEZE)	Years	Unlikely	$	$	$$
Secretions	Fluids	Scent marking glands	Dry swabs can be moistened with physiological secretions and can be stored in the field for long periods				$	$	$$
	Cytology	Female reproductive cycles	Vaginal dry swabs and stained smears to determine reproductive status	Room Temperature	Fixed smears - months	Easy	$	$	$
Ectoparasites	Parasitology	Ectodermic agents	Plastic container with 70-95% ethanol. For intradermic paraites (mites etc), scrape lesions with a scalpel blade, and place the scrapings in lactophenol	Room Temp	Years	Possible	$	$	$

[10] RNA Later, frozen and dried. -Pathogen detection by DNA/ RNA extraction. Tissue samples of maximum size 0.5cm x 0.5cm can be preserved in RNA-later for –PCR and immunology (DNA/RNA and antibodies). Place samples from different organs in different tubes and label accordingly. Samples in RNA-later can be stored at room temperature up to 2 weeks. Beyond this they should be transferred to a fridge or freezer. Thawing does not affect the sample quality, and samples can be shipped at room temperature.
Specific PCR for best candidate pathogens (according to symptoms, region etc). General PCR screening for various pathogens (in case best candidate pathogens are negative and for the detection of further pathogens). Cultivation of viruses and bacteria followed by microscopy or electron microscopy and/ or PCR. Further molecular methods. Antibody detection methods (ELISA, Western blot, immune histology etc.
10% glycerine (or similar) Pathogen detection by: culture of bacteria, characterisation of bacteria (classic bacteriology and molecular methods) Pieces should be very small, maximum 0.3 X 0.3 cm. Use different tubes and label accordingly. Samples can be stored for about 2 weeks at room temperature, later they should be stored frozen. Sample shipment is possible at room temperature.
[11] Tissue samples of maximum size 0.5cm x 0.5cm can be preserved in 10% formalin and used for cellular structure (histology and histopathology). You can pool samples from different organs in one pot of formalin. Make sure you use (at least 1Volume sample : 10 Volume Formalin). Samples preserved in Formalin are non infectious. You can also preserve faeces in 10% formalin for parasitology, in a separate container to the tissue samples.

Table 1 above summarises the biological samples that can be taken from an anaesthetised NHP, the information that they can yield, and appropriate storage methods. All samples should be carefully labelled (using a solvent-proof pen) with the date, location, species, individual identification, and the type and number of the sample taken.

SAMPLING DEAD ANIMALS

Dead NHPs may be found in the field, brought to the scientist for a number of reasons, or may result from a failed capture procedure. Post-mortem examinations of wild NHPs yield valuable data about cause of death and the health of the individual prior to death. Very little information is available on the natural pathology of wild NHPs and field studies offer one of the only opportunities for complete pathological examinations. Necropsy methods have been described elsewhere (Rabinowitz, 1993; Munson, 2000). Necropsy technique is vital to obtaining useful samples. Researchers are encouraged to become familiar with a complete necropsy protocol, whatever their area of primate research.

The person performing the necropsy should wear a mask, gloves and protective clothing. This is especially important when a carcass is found in the field and no detailed information is available on the case history. Do not touch or necropsy any carcass found with symptoms of severe hemorrhagic diseases. In such cases, reports must be sent to the relevant authorities as soon as possible.

See the next section for more complete notes.

3.18 NECROPSY PROCEDURES

W. Boardman, S. Unwin (Certain templates based on material from NEZS)

NECROPSY LOCATION or ROOM

Procedure

- Protective gear i.e. overalls, gloves, boots and aprons should be used. **In field situations, if level 3 or 4 biosecurity measures are suspected to be needed, contact the** Great Ape Health Monitoring Unit or WCS field veterinary staff for further advice
- A disinfectant foot bath should be at the entrance to the post mortem room area.
- After each necropsy, the kit is washed and stored, the table is cleaned down and disinfected and the floor is hosed down, washed and disinfected each time
- The Necropsy room should be well stocked at all times with items in the checklist.
- A full set of Necropsy instruments should be available and in good condition.
- Labels, pens, labelling pens should be available.
- When cleaning the room, carcases should not be mixed with non biodegradable material. They should be separated and disposed of accordingly.

NECROPSY SAFETY PRECAUTIONS

Personal Safety

Because some diseases of primates can cause serious illness or death in humans, all carcases should be handled as if they were harbouring potentially dangerous diseases and precautions for personal safety should be exercised. Minimal protective clothing includes coveralls, gloves and a mask that covers the nose and mouth, rubber boots.
Overalls with a washable rubber apron and rubber boots is recommended with face mask, coveralls, and double gloves.

Samples Handling and Carcase Handling and Disposal

Diseased wildlife also should be handled to minimise exposure of other people and other animals.
There is a duty of care to ensure that observers know of the hazards and are provided with protective clothing (especially if they are asked to participate.)
All sample containers, swabs, blood samples should be handled by the dissector only and placed in a plastic box for transportation. The dissector should ensure that no leakage occurs.

The carcase maybe required for further research. The carcase should be doubled bagged in large heavy duty plastic bags for transportation. Any

tissues not required should be buried in a deep hole or incinerated and not left unattended.

Cleaning

The examination table and floors should be thoroughly washed and then disinfected.
All contaminated waste paper or plastic materials should be incinerated or disinfected and bagged for removal.
All instruments should be cleaned with soap and warm water to remove blood and tissues and then they should be disinfected
Necropsy boots, apron and overalls should be washed and cleaned
The external surfaces of any containers with samples should be washed and disinfected.

NECROPSY INSTRUMENTS

Most damage to instruments sustain relates to cleaning, particularly delayed cleaning.

- Clean with a soft brush (open joints and/or disassemble).
- Soak in detergent
- Rinse
- Dunk in Surgical milk
- Place on padded surface to drain
- Instruments are ready to use
- Do not use abrasives or steel wool on instruments
- Avoid metal to metal contact
- Avoid over loading, e.g. cutting tissues that are too hard or thick with scissors

CHECKLIST FOR NECROPSY

Protective Clothing

- Rubber Gloves
- Rubber Boots
- Rubber Apron
- Overalls
- Mask (to cover mouth and nose) and eye goggles/ face shield

Necropsy Equipment

- Curved knife for skinning
- Straight pointed knife for dissection
- Sharpening stone or steel
- Dissecting scissors (small and large)
- 15-20cm rat tooth forceps
- 15-20cm pointed forceps
- Scalpel handle and blades
- Hack saw or bone saw
- Small and large shears

- Chisel and mallet/hammer
- Panga for removing spinal cord
- Chopping board
- Alcohol for sterilizing instruments
- Oscillating saw (in the future)

Specimen Containers and Sampling Equipment

- 5ml and 10ml syringes
- 20G needles
- Many 3-500ml wide mouth tight fitting containers or plastic jars for tissues
- Serum vacutainer tubes
- CPT vacutainer tubes
- Microscope slides
- Microscope slide container – small
- Sterile culture swabs with transport medium
- Small universal bottles for fluid samples, urine or for parasites
- Permanent marker pens and pencils
- Labels, labelling tape and tags
- String
- Plastic ruler or measuring tape
- Plastic zip-lock bags
- Aluminium foil
- Plastic pipettes
- Absorbent towel

Transport Materials

- Insulated cooler box with ice blocks
- Plastic box for transporting containers
- Sterile buffered glycerine (50%)

Fixatives

- 10% buffered formalin
- (Make by diluting 100ml 37% formalin with 900ml water. Add 4g sodium phosphate monobasic and 6.5g sodium phosphate dibasic)
- 100% acetone for cytology
- 70% ethyl alcohol for parasites
- Decal Solution (Mix 500mls formic acid (88-91% pure) with 4.5L buffered formalin).
- Bouins Solution (if necessary)

Disposal, Cleaning And Disinfecting Materials

- Plastic container or large thick plastic bags with string to hold carcase or parts of the carcase to move to Medical Waste Hole
- Rubbish bags
- Sharps containers
- Plastic bucket and brush
- Nailbrush, soap and towel

- Disinfectant – dettol
- Sodium hypochlorite (0.5%)
- 70% ethyl alcohol for disinfecting instruments

Documentation

- Camera and film
- Notebook and necropsy worksheets
- Necropsy procedures and protocols
- Pen and pencils

PERFORMING A PRIMATE NECROPSY

Introduction

- This procedure should be read with the necropsy sampling procedures below before the necropsy is performed
- All primates that die should be necropsied as soon after death as possible to establish the cause of death and to generate reference material for later study. It is vital that infectious diseases are recognised promptly and appropriate action taken to safeguard any in-contact primates.
- All necropsies should be done in a thorough, consistent and systematic way.
- All equipment should be available (see checklist) and all samples containers are labelled and readily accessible.
- Normally one or two people help with the dissection because a thorough dissection may take 3 hours.
- Store the body refrigerated at 4^{o}C until necropsy where this can be performed within 72hrs of death. If a fridge is not available keep the body as cool as possible.
- Do not freeze as ice formation within tissues considerably reduces the value of subsequent histological examination.
- When it is not possible to carry out a necropsy within 72hrs, the body should be frozen to arrest decomposition. Although histological examination then becomes difficult, at least gross lesions will still be identifiable once the body is thawed.
- In many cases bacterial cultures of tissue samples will still have value. Once thoroughly frozen at -20^{o}C or below, the body will be preserved for many months. Thawing may take longer than one might expect - allow at least 1 day for a adult female and up to 2 days for an adult male. Carrying out a necropsy on a frozen and thawed body is far from ideal and all attempts should be made to perform the necropsy before freezing is necessary.
- Photographs and or video recording of the necropsy are important for future reference. Photographs should be taken with a blue non reflective back ground and the sample/body should be labelled (to include date, species, ID, comparative measurement)
- All tissues sampled should be taken in duplicate as a minimum for possible future use. One set is sent for histopathology and the other set is kept for future reference. Representative samples of all tissues are collected and this includes apparently normal tissues and any abnormalities particularly with adjacent normal tissue.
- Any abnormalities should be described in the present tense using full sentences. The following criteria should be used

- Location
- Number and distribution
- Colour
- Shape
- Size
- Consistency and texture

History

- Note any accounts of the longevity of any illness.
- Read medical notes if available
- Note the ambient temperature and recent weather conditions
- Note any signs of struggle

External Examination

- Weigh the animal and record.
- Note any wounds. If present, look for bruising and bleeding in the tissues near the wounds which would indicate that they occurred before the animal died. Note any broken bones, broken or missing teeth
- Estimate the time of death and record.
- Note the muscle mass and condition of the chimpanzee and possible hydration status.
- A thorough visual examination is made of the entire body including all body orifices and any changes noted. The oral cavity should be examined and the condition of the teeth should be recorded. The condition of the skin and hair is noted and any signs of injuries/trauma to the head and body
- The extent of any external parasites should be noted and samples collected in 70% alcohol.

Internal Examination

- The animal is placed in the on its back (supine position) on the examination table and all four limbs are disarticulated by severing the muscular attachments in the axilla and inguinal regions.
- Viscera can be weighed and measured.
- A ventral midline incision is made from the pubic region to the mandibular symphysis. Note mammary glands, prepuce, penis and testes.
- Reflect the skin to the level of the backbone on right and left sides.
- Open the abdominal cavity along the ventral midline.
- Remove the entire ventral abdominal wall musculature from the lateral aspects of the lumbar vertebrae, dissecting carefully and take note of any abnormalities associated with the abdominal viscera. Take culture samples at this stage and any smears
- Using bone shears open the chest cavity along the backbone and working towards the cranial chest cavity and then continuing on the other side but this time working cranially - caudally. If a cosmetic necropsy be required then the dissection can continue at the costochondral junction on each side or along one side of the sternum being careful not to damage or contaminate the thoracic viscera.
- Remove the ventral chest wall, cutting through the diaphragm and take note of any abnormalities associated with the thoracic viscera. Bacterial cultures should be taken at this stage. Blood from the right side of the heart for later serology and for blood culture should be done at this stage.
- Locate the entry of the oesophagus into the stomach and ligate with string twice. Cut between the two ligatures.

- Remove the stomach and intestines as a unit by detaching the mesenteries from the intestines. Examine and sample any lymph nodes in the mesenteries.
- The pancreas and spleen remain attached to the stomach-intestine unit.
- Cut across the rectum ensuring no faeces fall into the abdominal cavity.
- Separate the bones of the larynx and dissect out the tongue, larynx and trachea and oesophagus and continue to work caudally towards the thoracic inlet. The entire length of the trachea and oesophagus unit and the lungs and heart to the attachment of the vessels and oesophagus as they go through the diaphragm are removed. Ligate the great vessels as they enter the diaphragm if necessary.
- The trachea is dissected from the larynx caudally to the end of the bronchi.
- The cervical and thoracic oesophagus is opened its entire length
- The heart is examined and weighed. Note amount of fat around the heart. The heart is dissected to examine all chambers and all the great vessels are opened to check for atheroma deposits
- Mediastinal lymph nodes are located, examined and sampled.
- The lungs are examined and dissected. Bacterial culture samples are taken from any abscesses and smears made.
- The head is disarticulated from the vertebral column and examined. The eyes, ears and nasal cavity are examined.
- The skin is removed from the head and the temporal muscles removed so that it easier to remove the cranium. The lines to cut are outlined in order to remove the brain with minimal disturbance. The cranium is removed carefully to reveal the meninges and brain. The surfaces are examined and bacterial cultures and smears are taken immediately. The dura mater is removed and the tentorium cerebelli is cut. The brain stem is severed and the cerebellum and the cranial nerves are severed using gravity to help reduce damage to the brain. The brain is removed and cut mid sagitally; one half is taken for histopathology and the other half can be taken for toxicology/virology.
- The skull is sawed mid-sagitally to examine the nasal cavity, turbinate bones and sinuses. The pharynx is also examined and a clearer view of the tonsils and teeth can be obtained.
- The obturator foramina are located and are dissected. The ischial bone is the then sawed on both sides into the obturator foramina. This piece of bone is removed and the pelvic canal is fully exposed. This allows full access to the reproductive tract which can be removed and examined.
- The mucosal and serosal surfaces of the reproductive tract are examined. Samples are taken.
- The adrenal glands are located and examined and transverse samples are taken. The kidneys are located and examined and samples taken of the cortex, medulla and pelvis.
- Joints are examined particularly the stifle and hip joints.
- The spleen is removed form the stomach and examined by slicing across in many sites
- The liver is detached and examined by cutting across in several sites. The hepatic lymph nodes are located and examined. The gall bladder is opened along the length of the bladder and into the bile ducts
- Skeletal muscle is taken from several places if possible including the thigh.
- A long bone preferably the femur is dissected and cracked open. The bone is placed in “decal” solution and the bone marrow is removed.

- Finally, the stomach and intestines are examined. Note the amount of ingesta in stomach and intestinal tract. The pancreas is located, examined, and sampled.
- Then the mesentery is cut along its entire length to allow the intestinal tract to be stretched out and examined more thoroughly. The stomach is opened and the surfaces examined. Note and describe the contents of the stomach and intestinal tract. Some of the contents may be kept for toxicology.
- The entire length of the intestinal tract is opened and examined. Samples are taken at various sites and parasites are removed and stored in 70% alcohol.

DEATH AND POST MORTEM EXAMINATION SUBMISSION FORM PLEASE COMPLETE ALL SECTIONS BELOW	
Free range/ large enclosure/ small enclosure:	Permanent I.D : Other I.D :
Species :	Sex :
DOB or Approx. age:	Enclosure I.D:
Number of this species remaining in enclosure/ area: (record as estimate if necessary)	Any other species in enclosure/ area: If yes what are they?
Circumstances of death or reason for euthanasia: Date died/euthanased: / / Found dead? Euthanased? Predated?	
Previous health status (tick one): 1. Previously healthy? 2. Under treatment? 3. Long term health problems? 4. Disease / deaths in group?	
Breeding history (tick one): 1. Offspring produced before 2. Mated but never produced offspring 3. Opportunity to but never seen mating 4. No opportunity for mating 5. Unknown	
Other Helpful Comments (PTO if required): Any recent husbandry changes or problems? (new introductions / moved / feeding / supplements / lighting / heating)?	

GROSS PATHOLOGY
Pathology Case Number:
Specimen Died:
Submitted by:
Death was spontaneous/ following acute illness/ following chronic illness
Enclosure/ location:
Clinical History:

Prosector: Necropsy Date:
Death to Necropsy Interval: 0-6 hours, 6-24 hours, > 24 hours (estimate time delay)
Necropsy Location:
Carcass Disposition: incineration/ burial – be as specific as possible:

AGE: Newborn/ baby/ juvenile/ adult / old adult weight :

MORPHOMETRICS:

STATE OF PRESERVATION: Good/ Fair/ Autolysed (Mild)/ Autolysed (Severe)

SYSTEMS
SKIN / APPENDAGES / EXTERNAL EXAM:

SENSORY:

MUSCULAR:

SKELETAL:

DIGESTIVE:

LIVER:

RESPIRATORY:

CARDIOVASCULAR:

LYMPHO RETICULAR:

URINARY:

ENDOCRINE:

REPRODUCTIVE:

NERVOUS:

Comment
Gross PM Key words (in order of importance): (Lesion/disease process, Topography, Topography/lesion/process modifiers, Severity, Chronicity
I.E- Distribution: Organ(s) – unilateral/ bilateral; focal/ multifocal; locally extensive/ diffuse
Whole Body – Localised/ generalises
Time: peracute/ acute/ subacute/ chronic/ chronic active
Severity: minimal/ slight/ moderate/ severe/ marked
Cause: Verminous/ bacterial/ chemical/ viral/ trauamatic/ protozoal/ mycotoc/ toxic etc/
Type: Croupous/ Haemorrhagic/ Purulent/ Necrotic/ Fibrinous/ Fibrinopurulent etc.
Laboratory tests requested/to follow: E.G Bacteriology and Parasitology
Samples Stored: E.G in formalin, frozen and as blocks/slides

NECROPSY SAMPLING PROCEDURES (refer to Section 3.17)

Histopathology

Soft tissues

- Representative samples are taken from all major organs and any abnormal tissues. Two sets of tissues are collected.
- Samples should be no larger than 10mm thick so that they can fix properly.
- Samples should be grasped carefully at the edges. Sections from hollow viscera or skin can be stretched flat on paper before being placed in the fixative container with the paper which ca be labelled.
- Most tissues are placed in a common container in 10% buffered formalin. Samples of lymph nodes ca be placed in separate containers and labelled.
- All tissues should be submerged in at least 10 times the volume of formalin as the volume of tissues collected.

Tissues contained bone

- Tissues that contain bone should be added to 10% formalin and then decalcified in "Decal Solution"
- Tissues should be submerged in "Decal Solution"in at least 20 times the volume of tissues collected.
- Renew the solution every third day and cut specimens into smaller pieces when possible.
- Place into 10% buffered formalin when tissues are readily sectioned with a scalpel blade.
- The container should be labelled with the Sanctuary ID, animals ID, age, sex, and date using a permanent marker pen or label.

Bacteriology

- Attempt to take samples without contaminating them. Take samples before touching the tissues and use sterile instruments (usually immersed in alcohol and flame them until red hot using a burner).
- Samples can be taken using a culture swab or sterile syringe and needle and placing contents into transport media or by placing a large tissue sample in a sterile container.
- Take samples from the edge of a potentially infected area where the bacteria are more likely to be alive.
- If there are no obvious signs of infection then samples from standard locations are suitable i.e. lung, liver, tonsil, kidney, spleen and intestines.
- Blood can be taken using a syringe and needle and removing any blood from the right side of the heart and dropping the blood onto the tip of the culture swab.
- Keep all samples moist using transport media, sealed and kept cool. If refrigeration is not possible then samples can be kept in buffered glycerine.
- Smears of pus or infected tissues are useful and can be air-dried, fixed in methanol, labelled and sent with other culture samples to the laboratory.

Virology

- Small segments of tissue i.e. liver, lung, heart, kidney, and any tissues with suspected lesions can be wrapped in aluminium foil, labelled and frozen.
- Whole blood can be stored in CPT tubes.

- Tissue samples can be stored in RNA Later

Toxicology

- Small segments of tissue i.e. liver, lung, heart, kidney, and any tissues with suspected lesions can be wrapped in aluminium foil, labelled and frozen.
- Contents of stomach can be kept in a small zip lock bag, labelled and stored frozen.

Serology

- Blood can be taken from the right side of the heart. If not clotted yet, then it can be allowed to clot and then spun down to remove the serum which is kept in a labelled plastic vial. If no centrifuge is available then the blood can be allowed to settle and the serum/plasma removed using syringe and needle and transferred into an EDTA tube or serum tube.
- Serum from whole blood can be removed by turning the vacutainer container upside down until it clots and the clot attaches to the rubber stopper. The vacutainer is then turned the correct way up and the rubber removed with the clot leaving serum in the tube which can be decanted to smaller vials and labelled.

Parasitology

- Make three blood smears and air dry
- Collect helminths in 70% alcohol or 10% buffered formalin
- External parasites can be collected in 70% alcohol.
- Faeces is stored in 10% buffered formalin

Cytology

- Make a clean cut of the tissue required and take a sample
- Grasp the sample and blot on a paper towel until no blood or clots are noticeable
- Gently touch the blotted surface on clean slides several times.
- Air dry slides and label

FIXED TISSUE CHECKLIST

Preserve the following tissues and samples of all lesions in 10mm segments in 10%buffered formalin.

1. Skin – full thickness abdominal skin, lip
2. Mammary gland
3. Salivary gland
4. Oral pharyngeal mucosa and tonsil
5. Tongue
6. Trachea
7. Larynx including air sac
8. Thyroid gland
9. Parathyroid gland
10. Lymph nodes – cervical, mediastinal, bronchial, mesenteric, popliteal, axillary, inguinal
11. Thymus
12. Lung – sections from several lobes and bronchi
13. Heart – Section including atrium, ventricle and valves from R & L heart.pericardium
14. Great vessels – especially pulmonary artery and coronary artery
15. Liver – sections from 3 different areas
16. Gall bladder and bile ducts
17. Spleen – cross section including capsule
18. Oesophagus – 3 cm long section
19. Stomach – 3 cm long section
20. Duodenum – 3 cm long section
21. Ileum - 3 cm long section
22. Caecum - 3 cm long section
23. Colon - 3 cm long section
24. Rectum - 3 cm long section
25. Omentum – 3cm square
26. Pancreas – section from two areas
27. Adrenal gland - transverse incision
28. Kidney – cortex, medulla and pelvis of each kidney
29. Bladder, ureters, urethra – cross section of bladder and sections of ureter and urethra
30. Uterus, ovary, cervix, vagina
31. Testes – transversely cut section
32. Prostate – transversely cut section
33. Eye
34. Brain – cut longitudinally along the midline
35. Spinal cord – sections from various sites
36. Diaphragm
37. Skeletal muscle – cross section of thigh muscle
38. Opened rib or longitudinally section of femur – marrow must be exposed for proper fixation
39. Joint tissues
40. Nerve tissue – brachial plexus
41. Neonate – umbilical stump.

42. Long bone - 1/2 of a femur including growth plate unless skeleton is required for other purposes.

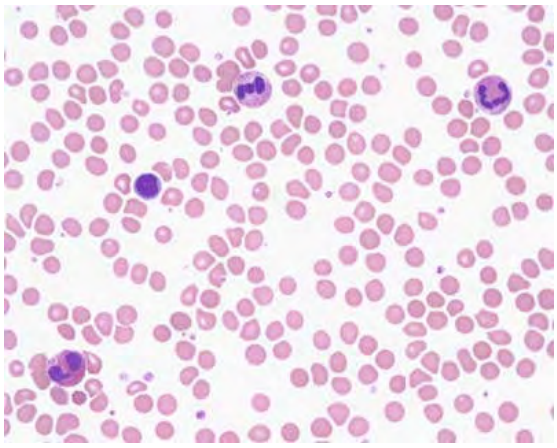
Tissue Checklist for Microbiology and Toxicology

Tissue	Microbiology	Toxicology
Brain	**X**	**X**
Fat		**X**
Kidney	**X**	**X**
Stomach contents		**X**
Hair	**X**	**X**
Liver	**X**	**X**
Whole blood	**X**	**X**
Lymph nodes	**X**	**X**
Tonsils	**X**	**X**
Spleen	**X**	**X**
Abscesses and granulomas	**X**	

3.19 CLINICAL PATHOLOGY FIELD BASICS

Compiled by S. Unwin (Reviewed by Mike Waters, Royal Veterinary College)

A. Haematology.



Figure

The haematology section is split into 2 parts. The first provides background information on blood components. This is very basic, and by no means complete but should be a good starting point for reference. The second part is a practical step by step guide on conducting a haemogram. Most conditions to be seen on a blood smear and discussed in part 1 can be visualised in part 2.
To do a haemogram accurately you will need a microscope with 100x capability, slides, a diff quick stain or similar, a microhaematic centrifuge and a refractometer. This may seem a lot but non of this relies on a viable electricity source, and can all be done rapidly, in an emergency situation. 98% of the work involves taking a decent blood smear and being able to interpret what you see, or at the very least, forwarding it on to someone who can interpret it for you.. For reference, review Veterinary Haematology (Schalm, Jah, Benjamen).
Blood biochemistry is not covered in this section. However, interpretation of biochemistry parameters can be undertaken with most veterinary texts, in combination with blood normals. ISIS (international Species Information System) values of blood normals for several PASA species are provided at the end of this section.

PART 1. Background information.

Introduction

Blood is one of the largest organs in the body, comprising 5-10% of total body weight. It consists of a variety of cell types bathed in a specialised fluid, the plasma, and its composition tends to reflect the health of an animal. In normal animals the cellular and biochemical components of blood are closely controlled within certain limits, but characteristic alterations occur in many disease states. Many of these changes are not specific but they can still provide a clue as to the nature of the disease process and can assist a clinical diagnosis. Blood is undoubtedly the most frequently sampled organ in sick animals, not just because it can be readily collected and evaluated, but because of the value of information it contains.

Cellular Components of Blood.

The major cell types present in the blood of most mammals are as follows:
Erythrocytes (red cells)
Leucocytes (white cells)
-granulocytes (neutrophils, eosinophils, basophils)
- lymphocytes
- monocytes
Thrombocytes (Platelets)

Immature erythrocytes and leucocytes can be found in the blood in some situations and their appearance may have diagnostic significance. Details will be discussed later.

The **haematopoietic system** which manufactures the cellular components of blood, is widely distributed throughout the body and includes organs which have functions other than blood formation and destruction. Organs either directly or indirectly involved in haematopoiesis are listed below.

- **Bone Marrow –** main source of erythrocytes, granulocytes, monocytes and thrombocytes. Also stores iron for heme synthesis and is the source of stem cells for T and B-lymphocytes which are produced at other sites. In young animals the marrow of all bones is actively producing blood cells but, with maturity, much of the haematopoietic (red) marrow is replaced with fat (yellow marrow). Active haematopoiesis becomes confined to the ribs, sternum, vertebrae, skull and pelvis.
- **Liver –** major haematopoietic organ during the first half of embryonic life. This function is gradually overtaken by the bone marrow but even in adults the liver retains its embryonic potential for haematopoiesis. The liver also stores iron, folic acid and vitamin B12; produces prothrombin, fibrinogen and albumin; converts free bilirubin to conjugated bilirubin for excretion in bile, and produces the precursor of erythropoietin.
- **Lymph Nodes –** produce T and B-lymphocytes, plasma cells. Participate in antibody production.

- **Spleen -** produces T and B-lymphocytes and plasma cells, stores erythrocytes and iron; removes aged and abnormal erythrocytes from circulation (blood filter), degrades haemoglobin
- **Thymus –** controls differentiation of bone-marrow derived stem cells and T-lymphocytes.
- **Tissue macrophage (reticuloendothelial) system –** lines sinusoids in spleen, liver, lymph nodes, bone marrow. Destroys aged or abnormal erythrocytes; degrades haemoglobin to iron, globin and free bilirubin; stores iron
- **Stomach and Intestinal mucosa –** HCl from stomach releases iron from organic complexes prior to absorption; small intestinal epithelium controls the rate of iron absorption in relation to body requirements; intrinsic factor from stomach required for Vit B12 absorption.
- **Kidney –** responds to hypoxia by producing an erythropoietic factor. This combines with an inactive plasma globulin of liver origin to form erythropoietin (stimulates erythropoiesis)

Primitive (pluripotential) stem cells in the bone marrow are capable of differentiating into committed (unipotential) cell lines which form each of the blood cell types.

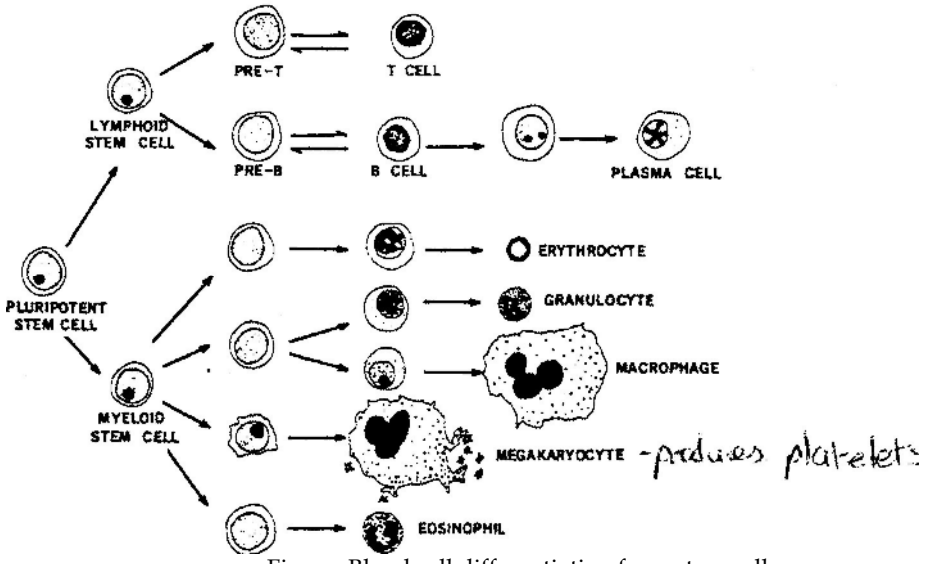


Figure Blood cell differentiation from stem cells

Figure above indicates blood cell differentiation from stem cells. It is NOT drawn to scale.

All blood cells (except for lymphocytes) have a limited life span and are incapable of self renewal. The bone marrow must therefore produce a steady supply of replacements, but a reserve of mature cells is normally held in the marrow ready for release in case of sudden demand.

Erythrocytes (Red Blood Cells)

ERYTHROPOIESIS

Erythropoiesis occurs extravascularly in the bone marrow in erythroplastic islets around central macrophages (nurse cells). Over a period of 3-4 days nucleated erythroid precursor cells progress through a series of divisions and maturation processes that result in the production of 8 to 16 rubricytes from each rubriblast. Metarubricytes are the final nucleated stage in erythroid maturation and once the nucleus is extruded reticulocytes are formed. Reticulocytes remain in the marrow for a further 2-3 days before being released into circulation where final maturation occurs. It takes approximately 5 days from the time of initial stimulation of stem cells by erythropoietin until reticulocytes are released from the bone marrow.

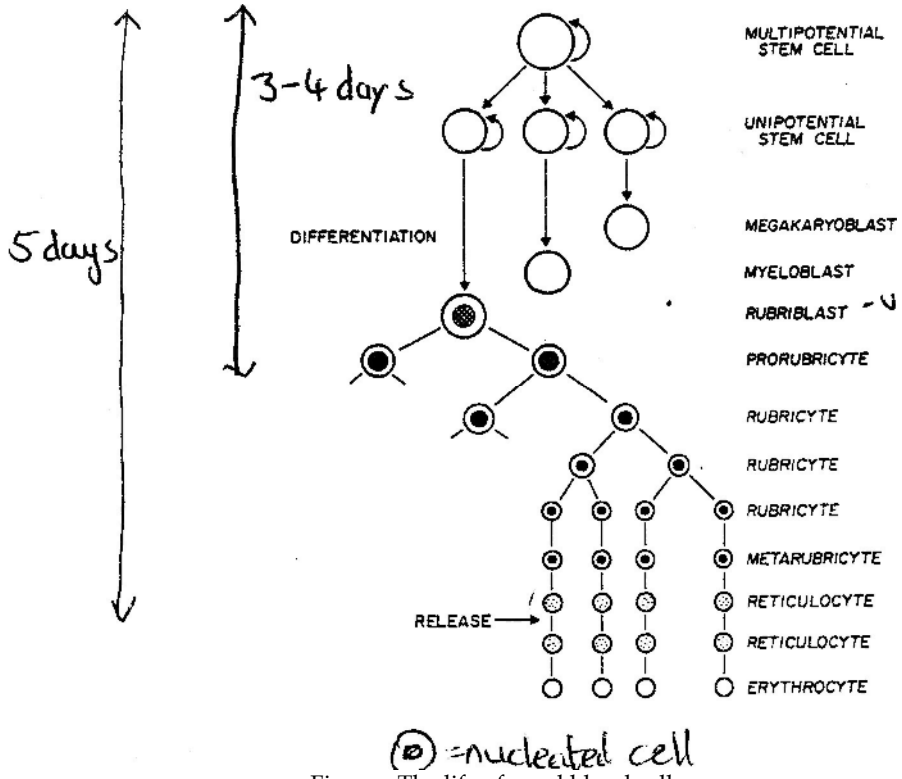


Figure. The life of a red blood cell.

Erythrocyte production can be accelerated several fold if the demand is great (eg. Anaemia), providing the necessary nutrients are available and the marrow is capable of responding.

Regulation of Erythropoiesis

Erythropoietin, produced in response to renal hypoxia, stimulates stem cell differentiation to rubriblasts in addition to promoting haemoglobin synthesis and early release of reticulocytes. It also reduces the maturation time of erythrocyte precursors. Macrophages in the bone marrow are probably involved in the short range regulation of erythropoiesis by producing erythropoietin.

Vitamin B12, vitamin B6 and folic acid are ESSENTIAL for erythrocyte production, as are the minerals iron, copper and cobalt. Deficiency of any of these nutrients, or of essential proteins and amino acids, can cause impaired erythropoiesis.

Androgens stimulate erythropoiesis while *oestrogens* have an inhibitory effect.

Erythrocyte Destruction

The life span of circulating erythrocytes is limited, but varies between species – approx. **120 days** in primates. Aged or damaged erythrocytes become les pliable and are removed from circulation by macrophages lining sinusoids in the spleen, liver and bone marrow. This process is called *extravascular destruction* or *haemolysis*. The spleen is best equipped to exert quality control standards on circulating erythrocytes as they squeeze through narrow gaps (3-5um diameter) in the walls of the sinusoids. Abnormal particles, inclusions, or organisms attached to the cells may be removed during this process, or the entire cell may be phagocytosed by the tissue macrophages.

Within minutes of phagocytosis haemoglobin is disassembled. Iron is released and transported via transferring back to the marrow for reuse in erythropoiesis. Bilirubin released from the broken protoporphyrin ring is carried by albumin to the liver where it is conjugated to glucuronide and excreted in the bile.

Intravascular haemolysis is a less important method of erythrocyte destruction. Haemoglobin released directly into the blood stream from lysed erythrocytes dissociates into alpha and beta dimmers which are rapidly bound to haptoglobin. The haptoglobin-haemoglobin complex is too large for renal excretion and is removed from the circulation by the liver where further processing occurs and iron and bilirubin are released.

If plasma haptoglobin is depleted during intravascular haemolysis unbound haemoglobin will be filtered by renal glomeruli. Much of it will be resorbed by renal tubular cells and converted to haemosiderin but in acute haemolytic diseases the capacity for tubular uptake may be exceeded and free haemoglobin will appear in the urine = RED WATER.

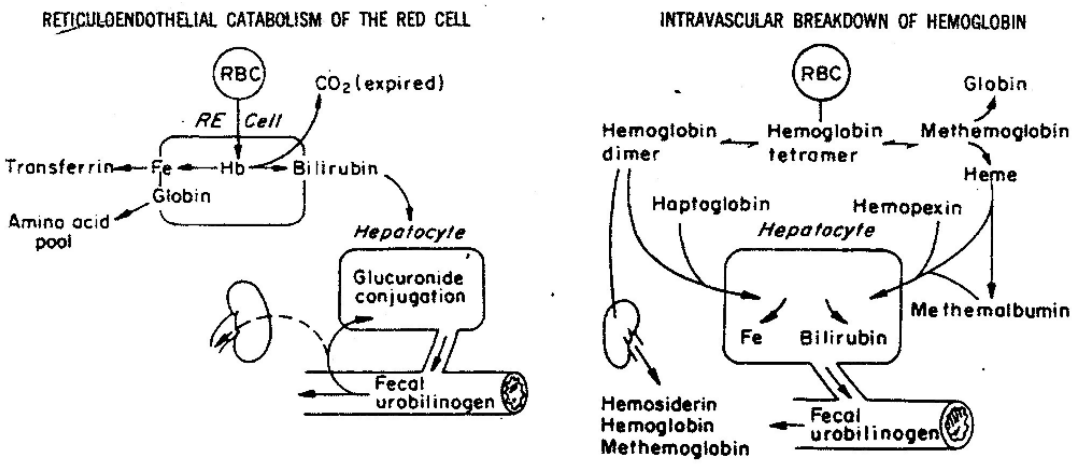


Figure. The processes of red blood cell breakdown

Iron Metabolism.

Iron is an essential component of haemoglobin and a continual supply is required by the bone marrow in order to satisfy the requirements for haemoglobin synthesis during erythropoiesis. In normal circumstances very little iron is lost from the body and the amount released from catabolised erythrocytes is almost enough to meet demand. Iron absorbed from the intestine provides the balance but this contribution is small, except in anaemic animals where the efficiency of intestinal iron absorption increases. An increased demand for iron during accelerated erythropoiesis also stimulates the release of iron from tissue macrophages in the spleen, liver and bone marrow where it is stored as ferritin or haemosiderin.

The copper-dependent enzyme ceruloplasmin (ferroxidase) is required for successful mobilisation of iron from body stores and absorption from the gut. Copper deficiency can therefore be a limiting factor in erythropoiesis.

EVALUATION OF THE ERYTHRON

See part 2 for details.

- **PCV/ Haematocrit**
- **Haemoglobin concentration** – provides the most direct indication of the oxygen carrying capacity of the blood.
- **Red cell (erythrocyte) count** – little value on its own but allows calculation of other useful parameters (MCV, MCH)
- **Erythrocyte indices –** Useful in classification of anaemias
  - **Mean corpuscular volume (MCV)**

$$\frac{PCV \times 10}{RBC\ count\ (millions)} = MCV\ (femtolitres)$$

An increased MCV is termed *macrocytosis*. The most COMON cause of this is post sampling artefact. It also occurs in anaemias where there is interference with nucleic acid synthesis and inhibition of cell division (eg Vit B12 and folic acid deficiency)

Microcytosis (decreased MCV) occurs in iron deficiency anaemia. This is because an extra cell division occurs during erythropoiesis before the haemoglobin concentration reaches the critical level sufficient to stop division.

An animal with regenerative anaemia will have a transitory increase in MCV because of the increased number of reticulocytes in circulation.

- **Mean corpuscular haemoglobin concentration (MCHC) –** Expresses the amount of haemoglobin in a decilitre of red blood cells and is the most accurate of the indices.

$$\frac{\textit{Hb concentration} \times 1000}{\textit{PCV}} = \textit{MCHC (g/dL)}$$

MCHC is decreased during reticulocytosis and iron deficiency but can be artifactually increased by haemolysis (due to measurement of extracellular Hb) and by the erythrocyte shrinkage (due to high concentration of anticoagulant).

An MCHC above 37 g/dL is unlikely to be valid as there is a limit to how much Hb an erythrocyte can contain.

- **Mean corpuscular haemoglobin (MCH) –** Often calculated but provides little further information. It expresses the amount of Hb contained in the average erythrocyte.

$$\frac{\textit{Hb concentration} \times 10}{\textit{RBC count (millions)}} = \textit{MCH (picograms)}$$

- **Reticulocyte Count** – the degree of reticulocytosis can be used as a measure of bone marrow response and should always be estimated in anaemic animals. It requires examination of blood smears stained with supravital stains such as new methylene blue, which demonstrate the RNA of immature erythrocytes better than standard blood stains.

The cytoplasmic RNA of reticulocytes may be diffuse or focally distributed (basophilic stippling). The very large reticulocytes (twice normal size) produced in animals with severe anaemia are often called **shift reticulocytes**. In routinely stained blood smears reticulocytes appear as large, slightly basophilic erythrocytes but supravital staining is required for accurate counts. The presence of erythrocytes with varying staining affinity is termed *polychromasia.*

Reticulocytes are usually reported as a percentage of the red blood cell count BUT in anaemic animals this does not allow for the relative increase resulting from the reduced numbers of erythrocytes, or for the earlier stage at which reticulocytes are released from the bone marrow during responsive anaemias. A modest increase in reticulocyte percentage could therefore incorrectly suggest a regenerative anaemia. It is more useful to use either the absolute number of reticulocytes or a value corrected for the reduced number of erythrocytes.

- **Erythrocyte morphology** – a microscopic examination of a blood smear is an important part of any haemotologic examination. Not only is it necessary for differential leucocyte counts but abnormalities in erythrocyte morphology can provide valuable information on the cause of anaemia, and not the responsiveness of the bone marrow. It is necessary to first become familiar with the normal erythrocyte morphology in each species, and with common artefacts that could be misinterpreted.

CLASSIFICATION OF ANAEMIA

Anaemia is a reduction in erythrocyte numbers and/or haemoglobin concentration below normal, but a diagnosis of anaemia on its own is of limited value and an effort should always be made to determine the cause. The first step is to classify the anaemia into either regenerative (bone marrow responsive) or nonregenerative types.

In **regenerative anaemia** the erythroid marrow responds to the reduced cell mass by accelerating erythropoesis. This can be detected in the peripheral blood by the presence of increased numbers of reticulocytes, macrocytosis (increased MCV), polychromasia, basophilic stippling (occasionally), and sometimes nucleated erythrocytes.

Regenerative anaemias are caused by either blood loss or haemolysis.

Nonregenerative anaemia occurs when the bone marrow cannot produce enough erythrocytes to replace those which are lost by normal attrition. This may be due to deficiency of a factor required by erythrocyte production (eg. Iron), inhibition of the bone marrow by certain toxins, infections or neoplasms, defective maturation of erythrocytes, or to deficiency of erythropoietic stem cells. Bone marrow examination may provide a clue as to the cause.

Anaemias can also be classified according to the size and haemoglobin concentration of erythrocytes, ie **normocytic, macrocytic, microcytic** and either **normochromic or hypochromic** (increased MCHC is not physiologically possible). This system is useful for indicating iron deficiency and megaloblastic anaemias.

REGENERATIVE ANAEMIAS

1. Blood Loss

The peripheral blood picture in blood loss anaemias can vary markedly depending on the severity and rapidity of blood loss, whether it is internal or external, and the stage at which the sample was collected.

In **acute blood loss** all erythrocyte parameters (PCV, RBC count, Hb concentration, MCV, MCHC) will initially be NORMAL because the cellular and fluid components are lost in similar proportions, although the animal may develop hypovolaemic shock if the blood volume is reduced to 60-70% of normal. Splenic contraction delivers highly concentrated blood (PCV 80%) into circulation and may temporarily elevate the PCV, but as the blood volume is gradually restored by the addition of interstitial fluid the erythron is diluted and the PCV, RBC count and haemoglobin concentration are reduced. The plasma protein concentration will also be reduced.

Platelet numbers may increase during the first few hours after haemorrhage and neutrophilic leucocytosis commonly occurs by approximately 3 hours. Signs of increased erythropoiesis become evident in the peripheral blood 48-72 hours post haemorrhage and reach a peak at approximately 7 days but are preceded by erythroid hyperplasia in the bone marrow. The haemogram should return to normal in 10-14 days after a single haemorrhagic episode and if reticulocytosis persists for more than 2-3 weeks then continued blood loss should be suspected.

Causes of acute blood loss include:

- Trauma
- Surgery
- Gastrointestinal ulcers
- Coagulation defects

In **chronic blood loss** the anaemia develops slowly and hypovolaemia does not occur because the animal has time to adapt. The PCV may reach a low level before clinical signs of anaemia develop and at this stage there will usually be evidence of a regenerative response as well as hypoproteinaemia. This can therefore reduce to much lower levels without death, as the animal has time to compensate.

Persistent haemorrhage will eventually cause iron depletion and the anaemia becomes progressively less responsive. The serum iron concentration and percent saturation of transferring will be reduced and the anaemia may become microcytic and hypochromic.

When blood loss is internal (into body cavities) approximately two-thirds of the erythrocytes enter lymphatics and are recirculated within 24-72 hours. The remainder are lysed or phagocytosed but their iron is not lost, and there is no loss of plasma proteins, so the anaemia is not as severe as in external blood loss (including gastrointestinal haemorrhage), and the regenerative response is greater. A much greater regenerative response (reticulocytosis) however

occurs in haemolytic anaemia where iron from lysed erythrocytes can be utilised directly.

Causes of chronic blood loss include:

- Internal parasitism eg. Hookworms
- External parasitism eg. Ticks, lice
- Gastrointestinal ulcers
- Bleeding disorders eg. Haemophilia, thrombocytopaenia.

2. **Accelerated erythrocyte destruction (haemolytic anaemia)**

Haemolytic anaemia occurs when accelerated erythrocyte destruction is not balanced by increased erythropioesis. Icterus and/or haemoglobinuria may be observed clinically depending on the severity and mechanism of the haemolysis. As mentioned above, reticulocyte counts are usually higher in haemolytic than haemorrhagic anaemias. Another important distinction is the presence of normal or elevated plasma protein concentrations in haemolytic anaemia. Neutrophilic leucocytosis with a left shift (but without toxic changes) may occur. Abnormalities in erythrocyte morphology in peripheral blood smears may suggest the mechanism of haemolysis or even provide an aetiologic diagnosis.

Multiple causes of accelerated erythrocyte destruction have been described in animals and the site of haemolysis can be either intravascular or extravascular (phagocytic).

In many haemolytic syndromes erythrocyte destruction occurs at both intravascular and extravascular sites, but usually one site predominates.

- **Intravascular haemolysis** occurs when the erythrocyte membrane suffers sufficient damage to allow escape of haemoglobin into the plasma. Examples include:
  - Complement-mediated lysis (eg. Some types of immune-mediated anaemia)
  - Oxidative injury resulting in denaturation of haemoglobin to Heinz Bodies and lysis of erythrocytes (eg. Certain chemical and plant toxins).
  - Osmotic lysis due to erythrocyte membrane alterations or infusion of hypotonic fluid.

Laboratory results characteristic of intravascular haemolysis may include:

- Haemoglobinaemia, haemoglobinuria, haemosiderinuria (urinary iron)
- Increased MCHC (artifactual)
- Decreased serum haptoglobin concentration
- Hyperbilirubinaemia, but only if there has been time for bilirubin to be formed (at least 8-10 hours) and if liver capacity to remove the conjugate bilirubin has been exceeded.
- Alterations in erythrocyte morphology (eg. Heinz bodies, fragmented erythrocytes, erythrocytic parasites).
- High antibody titre to a specific agent (eg. Leptospira).

- **Extravascular haemolysis** involves accelerated removal of erythrocytes by phagocytic macrophages, especially those in the spleen. This may be the result of:
  - Reduced erythrocyte deformability due to membrane alteration (eg. Blood parasite, immune-mediated anaemias, microangiopathies)
  - Impaired glycolysis or low ATP content of erythrocytes (eg pyruvate kinase deficiency)
  - Immunoglobulins and/or C_3 attached to erythrocyte membranes (recognized by receptors on macrophages)

The clinical course is usually more chronic than with intravascular haemolysis and there is no evidence of haemoglobinaemia or haemoglobinuria. Hyperbilirubinaemia seldom occurs.
Splenomegaly (enlarged meaty spleen) may be a prominent feature due to increased macrophage activity and to extramedullary haematopoiesis. This can be readily palpated on a clinical exam.
Additional features of extravascular haemolysis may include abnormal erythrocyte morphology (eg. Erythrocytic parasites, spherocytes, fragmented erythrocytes).

TABLE: Examples of Haemolysis Causes

	Intravascular Haemolysis	**Extravascular Haemolysis**
Infectious	*Leptospira Pomona*	Malaria
	Clostridium haemolyticum	*Anaplasma*
	Babesia spp.	*Haemobartonella spp.*
Toxic	Copper (chronic)	
	Zinc	
	Paracetamol	
Immune Mediated	Autoimmune haemolytic anaemia	Autoimmune haemolytic anaemia
	Neonatal isoerythrolysis	
	Incompatible blood transfusion	
Inherited RBC defects		Pyruvate kinase deficiency
		Erythropoietic porphyria
Miscellaneous	DIC	DIC
	Haemangiosarcoma	Haemangiosarcoma
	Hypophosphoteamia	Vascular disease
	Selenium/ Vit E deficiency	
	Hypotonic fluid infusion	
	Excess water intake	
	Liver disease	

NON REGENERATIVE ANAEMIAS

Nonregenerative anaemias are associated with an abnormal bone marrow which is unable to produce adequate numbers of erythrocytes. Depending on the mechanism, other cell lines may also be involved, resulting in neutropaenia and thrombocytopaenia as well as anaemia (pancytopaenia).

Nonregenerative anaemias may be due to either reduced erythrocyte production or reduced haemoglobin synthesis.

1. **Reduced erythrocyte production**.

a. ***Hypoplastic and aplastic anaemia*** resulting from stem cell injury occurs in man and animals. Aplastic anaemia is generally characterized by pancytopaenia but selective marrow depression with pure erythroid aplasia has been reported.

Possible causes include:

- Cytotoxic agents eg. Chloramphenicol, anticancer drugs, radiation, oestrogens, phenylbutazone
- Viruses, eg. Some retroviruses
- Trichostrongyle nematodes

b. ***Myelophthisic anaemias*** occur when the bone marrow is extensively replaced by fibrous tissue (myelofibrosis), bone (osteopetrosis), infiltrating neoplasms (eg. Leukamias, metastatic tumours) or granulomatous inflammatory tissue (eg. Histoplasmosis)

c. ***Anaemia of chronic inflammatory or neoplastic disease*** results from sequestration of iron in the body's storage pool where it is unavailable for erythropoiesis. Increased quantities of iron-containing pigment (haemosiderin) are present in the bone marrow. Erythrocyte survival time is also reduced in anaemia of chronic disease.

d. ***Protein deficiency anaemia*** occurs in chronically starved or parasitized animals

e. ***Deficiencies of vitamin B12 and folic acid*** cause impaired nucleic acid synthesis and megaloblastic anaemia in humans (rare in animals)

f. Hypoplastic anaemias occur in some ***endocrine disorders*** eg. Hypopituitarism, hyperparathyroidism and erythropoietin deficiency.

2. **Reduced haemoglobin synthesis.**

a. ***Iron Deficiency*** is one of the most common causes of anaemia in animals. It occurs most often in association with chronic blood loss (eg. Internal parasites). Impaired heme synthesis in iron deficiency leads to microcytic, hypochromic anaemia. Iron deficiency may also be associated with increased fragility of erythrocytes and the appearance of fragmented forms in circulation

b. ***Copper Deficiency*** can induce an iron deficiency anaemia because of the essential role of copper in the enzyme ceruloplasmin (ferroxidase). This enzyme is required for the transfer of iron through gut epithelium into the blood and for the mobilization of iron from body stores.

c. ***Vitamin B6 (Pyridoxine) deficiency*** inhibits the production of the protoporphyrin component of haemoglobin and leads to the accumulation of iron within mitochondria, especially in erythrocyte precursors.

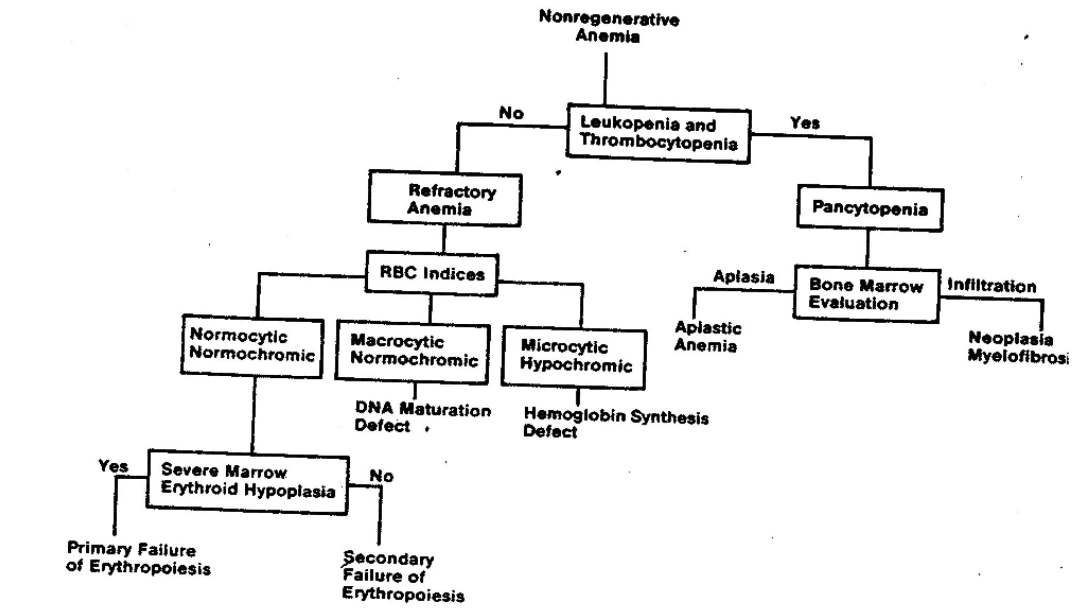


Figure 1—Problem-oriented approach to nonregenerative anemias in the dog.

Figure. Promlem oriented response to non regenerative anaemias in the dog.

POLYCYTHAEMIA

An increase in the PCV, erythrocyte count or haemoglobin concentration above the normal range is termed polycythaemia. This is USUALLY RELATIVE – ie, associated with DEHYDRATION. Primary polycythaemia is extremely rare, but secondary absolute polycythamia can be seen in chronic respiratory disease, high altitude, congenital heart defects and some tumours.

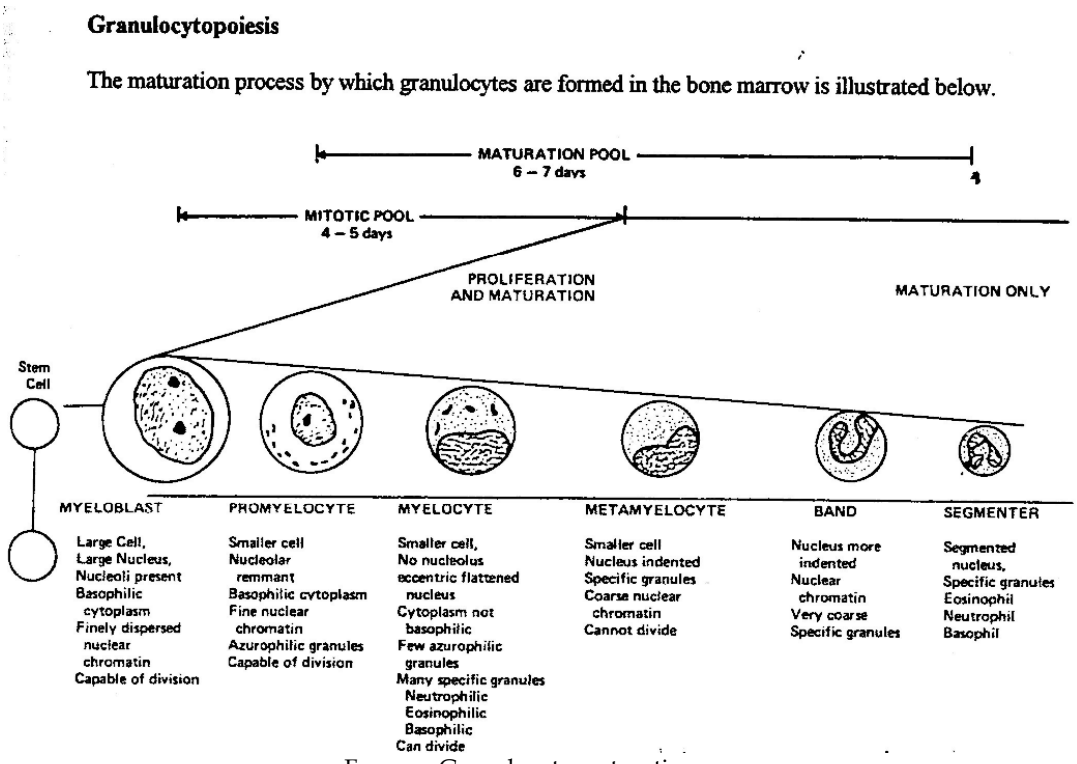
LEUCOCYTES (WHITE BLOOD CELLS)

Leucocytes are primarily involved in body defence mechanisms and are capable of countering a wide variety of different infectious agents or foreign material. The two basic mechanisms are phagocytosis and antibody production. Leucocytes with phagocytic properties include granulocytes (neutrophils, eosinophils and basophils) and monocytes. Lymphocytes are not phagocytic but are involved in antibody production and cell-mediated immunity. In spite of these differences the two systems often work together in defencse. For example, macrophages are required to "process" antigens for the B-lymphocytes which produce specific antibodies, and bacteria coated with antibodies produced by lymphocytes can be more effectively phagocytosed by neutrophils.

GRANULOCYTES

Granulocytopoiesis

The maturation process by which granulocytes are formed in the bone marrow is illustrated below.



Fugure. Granulocyte maturation

It normally takes 6-7 days for the production of mature granulocytes from the myelocyte stage. Mature granulocytes usually remain in the marrow for an extra 3-4 days before being released into the blood, but they can be released immediately on demand.

Extramedulllary granulopoiesis can also occur in the spleen, liver and lymph nodes if there is an intense, prolonged demand for granulocytes

Granulocyte Function

1. Neutrophils

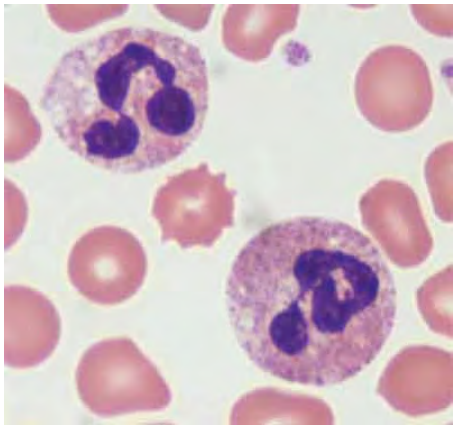


Figure Neutrophils - HUMAN

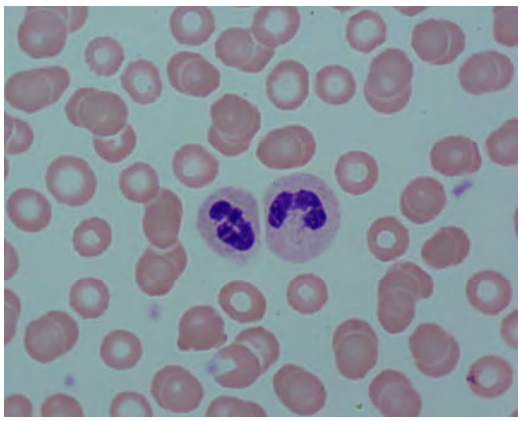


Figure Neutrophils – CHIMPANZEE

Neutrophils constitute the main cellular defense system against bacteria but can also damage or destroy fungi, viruses, algae and parasites. They are attracted to sites of infection by chemotactic factors (eg. Complement components C_3, C_5 and split products of the clotting system) where they phagocytose and destroy bacteria or other small organisms.

Phagocytosis is aided by opsinisation of the organism with IgG or complement (C_3, C_5). Once the organism is neutralised the phagosome in which it is contained fuses with primary lysosomes containing a variety of lytic enzymes. Highly reactive oxygeb radicals (eg. H_2O_2 and superoxide) are generated by a "respiratory burst" within the newly formed phagolysosome and the organism is killed. Neutrophils secrete endogenous pyrogen when exposed to bacteria or bacterial products and can therefore contribute to the fever associated with infections.

2. Eosinophils

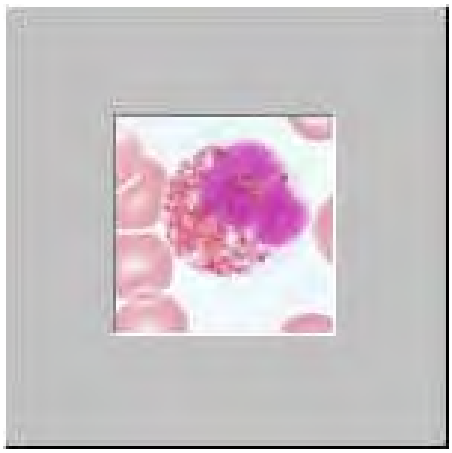


Figure Eosinophil – HUMAN

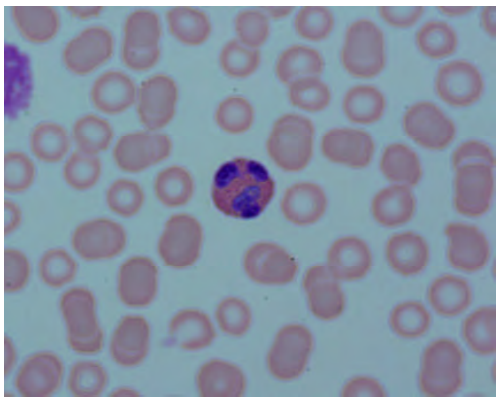


Figure Eosinophil – CHIMPANZEE

Eosinophils appear to be derived from a different stem cell to that which produces neutrophils and their production is controlled by T-lymphocytes. Their function is still poorly understood but they are attracted to sites of mast cell degranulation and can neutrolise substances (eg. Histamine) released during immediate hypersensitivity reactions. They also appear to participate in the defense against certain parasitic infections. They live about 2-3x longer than neutrophils.

Eosinophils are much more common in tissues than in blood (ratio approx. 300:1). Their cytoplasm is packed with eosinophilic granules and their nucleus contains fewer segments than neutrophils. Eosinophils are phagocytic, have bacteriocidal properties similar to neutrophils and respond to similar chemotactic factors.

3. **Basophils**

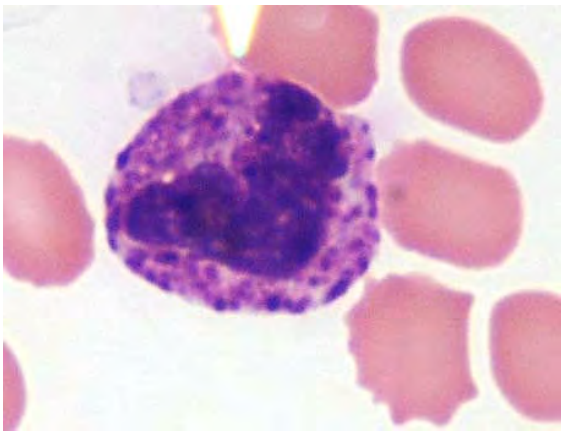


Figure Basophil – HUMAN

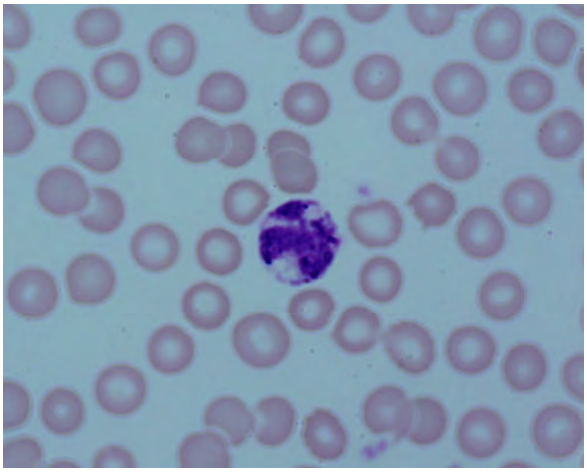


Figure Basophil - CHIMPANZEE

Basophils are closely related to tissue mast cells and are found in very low numbers in blood. Their cytoplasm contains multiple, large, blue/black granules which may obscure the nucleus. Basophils appear to play a central role in acute systemic allergic reactions. Their granules contain mediators of immediate-type hypersensitivity reactions (including heparin and histamine) as well as an activator of plasma lipoprotein lipase.

Granulocyte Kinetics

1. Neutrophils

In healthy animals neutrophils remain in the blood for an average of only 10 hours, all blood neutrophils being replaced approximately two and a half times each day. They move from the blood into tissues in a random fashion, not dependant on their age, and do not return to circulation. Many are lost through mucous membranes and in body secretions.

There are two major pools of neutrophils within the blood:

- *Marginal neutrophil pool (MNP)* – which consists of neutrophils loosely adherent to the vascular endothelium (and not included in total white cell counts)
- *Circulating neutrophil pool (CNP)* – consisting of neutrophils circulating freely together with erythrocytes and other blood cells. Neutrophil number, derived from routine white cell counts and differentials is an estimate of the CNP.

In primates, the 2 pools contain approximately equal numbers of neutrophils.

The relative size of the two neutrophil pools can be altered by a variety of physiologic or disease states and can have a marked effect on the neutrophil (and total leucocyte) count.

a. Neutrophilia (increased neutrophil count)

Three main causes of neutrophilia are recognised:

Physiologic neutrophilia occurs in response to the release of adrenaline in animals subjected to excitement, exercise, fear etc., and reflects mobilisation of neutrophils from the marginal pool. No immature neutrophils are released from the bone marrow and the response lasts only 20-30 minutes

Stress neutrophilia is induced by corticosteroid hormones. Corticosteroids cause increased transit time of neutrophils in circulation by reducing neutrophil adherence to the endothelium and therefore reducing the emigration of neutrophils from the blood. The release of neutrophils from the bone marrow is also increased but this does not include immature forms. Stress neutrophilia is typically accompanied by lymphopaenia and eosinopaenia (so-called "stress leucogram")

Neutrophilia of inflammatory disease. In the early stages of an inflammatory disease the demand for neutrophils in tissues may induce a short-term neutropenia due to rapid emigration from the blood. This is followed by increased release of neutrophils from the storage pool in the bone marrow and, providing the input of neutrophils exceeds emigration, neutrophilia will occur. The release of immature neutrophils (**band cells** – see next photo) from the bone marrow is an expected response to an inflammatory disease and is called a **left shift**. In severe inflammatory diseases the storage pool of neutrophils in the bone marrow may become depleted and both band cells and metamyelocytes may be released (marked left shift). Provided the left shift is accompanied by neutrophilia it is considered to be a **regenerative left shift.**

In cases where there is a prolonged or overwhelming tissue demand for neutrophils the left shift may be accompanied by a neutropenia. A marked neutrophilia accompanied by a severe left shift which includes metamyelocytes and myelocytes is sometimes called a **leukaemoid response** and usually reflects a serious inflammatory disease,

In general, localised infections such as pyometra incite a greater neutrophilic response than generalised infections, and pyogenic organisms such as bacteria cause a greater response than non pyogenic organisms (eg. Viruses, parasites).

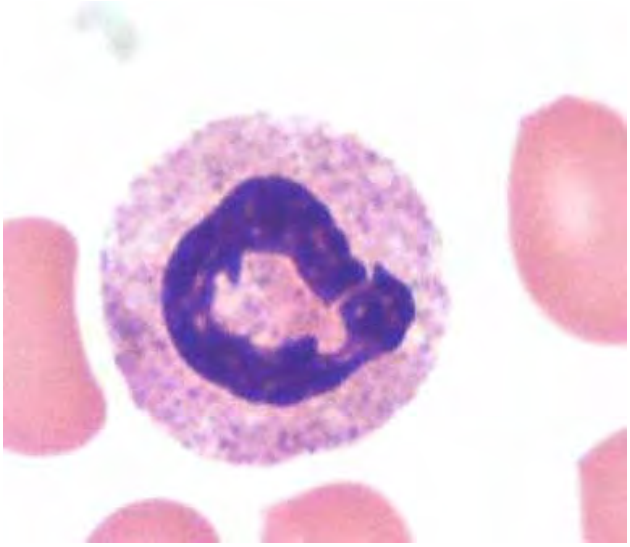


Figure Band cell – HUMAN an immature neutrophil. Don't confuse this with a monocyte – review pictures at the end of part 2.

b. Neutropenia (decreased neutrophil count)

Sequestration neutropenia is due to an increase in the proportion of neutrophils in the marginal pool. This occurs in anaphylactic shock and in response to endotoxaemia. Endotoxin induced neutropenia lasts only 1-3 hours and is followed by a mild neutrophilia.

Excessive tissue demand for neutrophils can cause marked neutropenia with a high percentage of immature neutrophils in circulation. The transit time in blood is reduced and the rate of emigration of neutrophils into tissues exceeds their rate of release from the bone marrow. Acute pyogenic bacterial infections are the main cause.

Not surprisingly, neutropenia (whatever the cause) is accompanied by an increased risk of bacterial infection.

c. Toxic Changes in neutrophils (see pictures in part 2).

Dohle bodies are pale blue or grey, irregular shaped cytoplasmic inclusions that occur in neutrophils during many infections or toxaemias. They result from incomplete cellular maturation and utilisation of RNA and reflect a mild toxic change.

Cytoplasmic basophilia (toxic granulation) is common during severe bacterial infections and reflects a high RNA content secondary to defective maturation. It occurs in neutrophils, band cells and metamyelocytes.

Cytoplasmic vacuolation often occurs with basophilia in severe septicaemic infections

2. Eosinophils

The blood transit time for eosinophils is 24-35 hours.

a. **Eosinophilia** occurs most often in situations where there are antigen/antibody (IgE) interactions with mast cells in tissues such as skin, lung or gastrointestinal tract. Parasites may be involved but not all parasitic infections will induce an eosinophilia, and it will only occur in animals that have previously been sensitised. The migration of parasitic larvae through tissues is likely to cause eosinophilia.

b. **Eosinopenia** is a well recognised response to increased blood concentrations of corticosteroids (exogenous or endogenous). The mechanisim may involve sequestration of eosinophils in capillary beds, especially in lymphoid organs. Corticosteroids also cause reduced degranulation of mast cells and since histamine from mast cell granules is a potent chemoattractant and release factor for eosinophils this may contribute to the development of eosinopenia Eosinopenia can also occur in acute inflammatory conditions, partly due to the effect of corticosteroids and adrenaline, but probably also due to increased migration of eosinophils to sites of inflammation

3. Basophils

Basophilia may occur in episodes of hyperlipaemia or may accompany eosinophilia in disorders of prolonged antigen/ antibody (IgE) stimulation such as chronic dirofilariasis.

MONOCYTES

Formation, distribution and function.

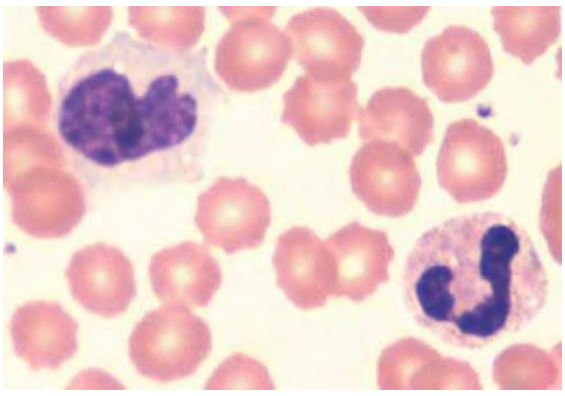


Figure Monocyte and neutrophil. HUMAN.

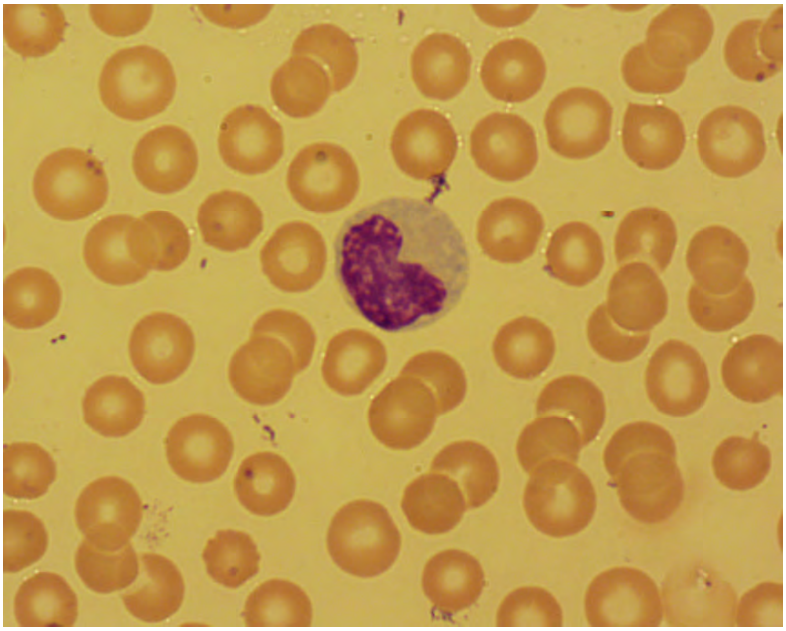


Figure Monocyte - CHIMPANZEE

Monocytes are formed in the bone marrow and share a common stem cell with neutrophils but have a shorter maturation process. They circulate in the blood for a short period then enter body tissues as either mobile or fixed **macrophages** capable of surviving for several weeks or even months. These tissue macrophages (eg. Kupffer cells in the liver, macrophages in inflammatory exudates, microglial cells in the CNS) are capable of multiplication and in some locations they develop specific functions.

The phagocytic properties of macrophages allow them to play an important role in defence against certain facultative intracellular bacteria (eg. Mycobacteria) as well as fungi. They also remove aged or injured cells and necrotic tissues, processs antigens for presentation to lymphocytes, store iron and synthesise several important substances (eg. Certain complement components, granulopoietin). The close association between macrophages in tissues can produces a **granulomatous reaction**.

Conditions capable of inducing monocytosis include:

1. *Tissue necrosis* with a high demand for phagocytes to remove necrotic debris
2. *Chronic infections*, particularly those associated with an on-going cell-mediated immune response
3. *Corticosteroid excess* in hyperadrenocorticism, stress or secondary to exogenous administration often causes monocytosis
4. *Neutropenia* caused by granulopoietic hypoplasia may also induce compensatory monocytosis

LYMPHOCYTES

Formation, Distribution and Function

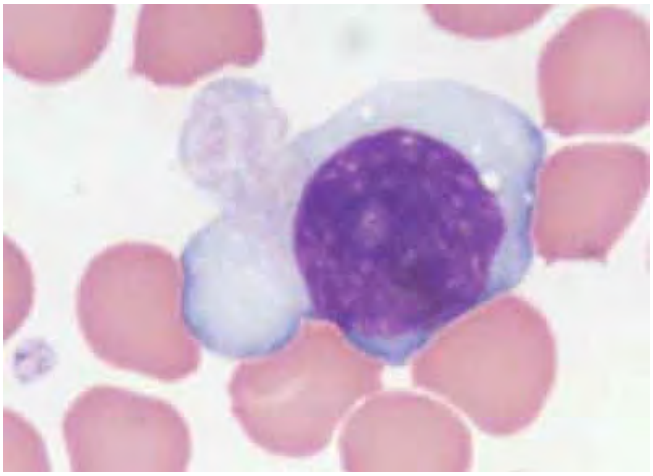


Figure Atypical (or squished) lymphocyte - HUMAN

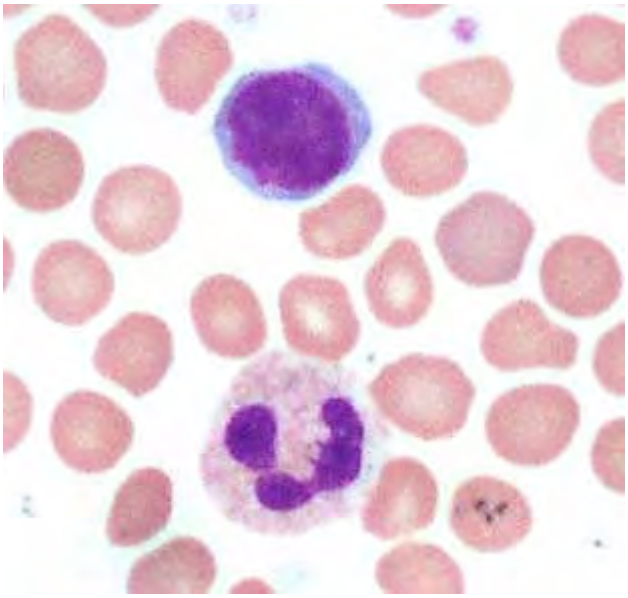


Figure Lymphocyte and neutrophil - HUMAN

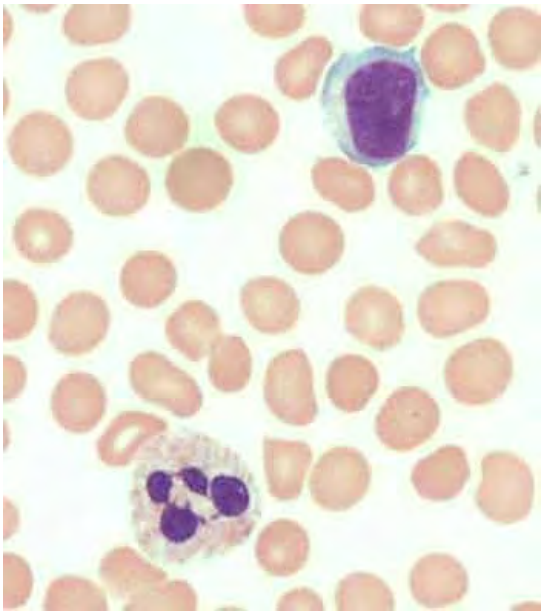


Figure Lymphocyte and neutrophil - HUMAN

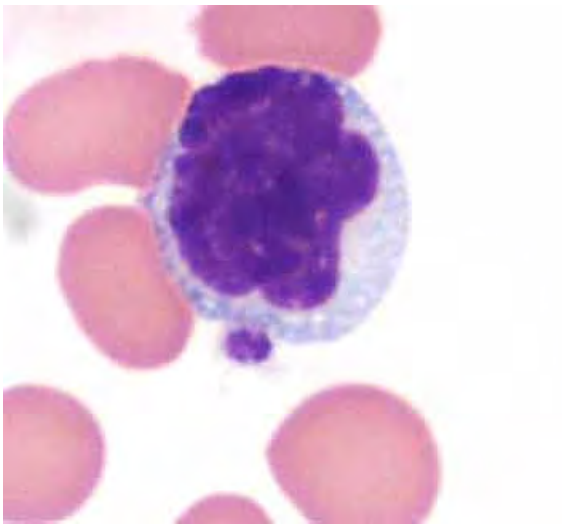


Figure Lymphocyte – HUMAN

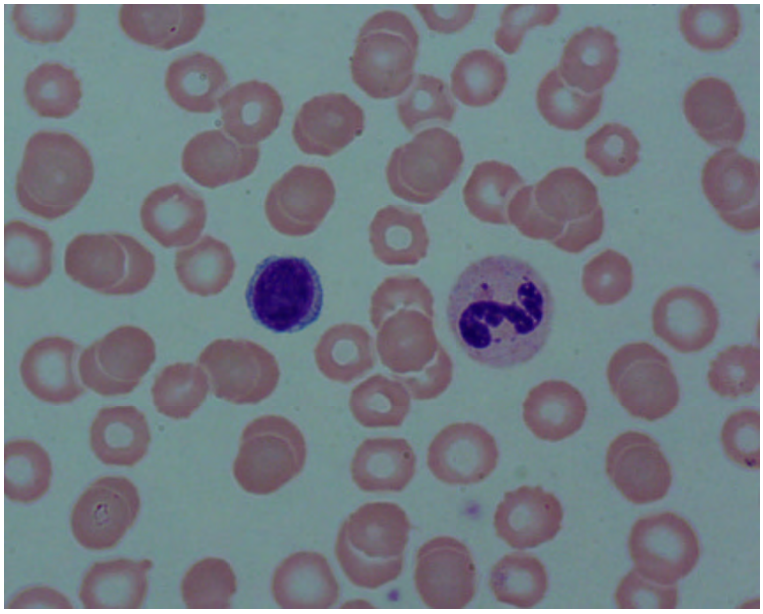


Figure Lymphocyte and neutrophil - CHIMPANZEE

Lymphocytes can be broadly classified into one of two groups. B-lymphocytes (involved in antibody production) or T-lymphocytes (involved in cell-mediated immunity). Stem cells of both types originate in the bone marrow but during foetal development there is migration of T and B-lymphocyte precursors to the thymus and gut associated lymphoid tissue respectively. T-lymphocytes are produced either in the thymus itself or in thymic dependant areas of the spleen and lymph nodes. B-lymphocytes are also produced in peripheral lymphoid organs, but in different areas than T-lymphocytes.

The control of lymphopoiesis is complex. Not only is there a primary intrinsic control mechanism but a secondary mechanism responsive to antigenic stimulus also operates. This secondary lymphopoiesis appears to occur mainly in the spleen, lymph nodes and gut associated lymphoid tissue although the thymus is probably the main site of lymphocyte production.

B-lymphocytes stimulated by exposure to specific antigens differentiate into plasma cells which are capable of producing large quantities of antibody.

T-lymphocytes have many functions. Not only are they cytotoxic for many virus infected cells, tumour cells and parasites but they are also involved in regulating B-lymphocyte function and influencing the production of granulocytes and monocytes. Lymphokines produced by activated T-lymphocytes are mediators of cell-mediated immune reactions.

Lymphocyte Kinetics.

Compared to other leucocytes, lymphocytes have a long lifespan. Some sub populations live for only a few days but others, probably committed memory cells, can live for months or even years. The blood contains less than 5% of the total body lymphocyte population.

A recirculating pool of lymphocytes travels via lymph nodes from blood to lymph and back again several times per day. In healthy animals the number of lymphocytes in the blood is relatively constant although it varies between species and in general, declines with age.

1. **Lymphocytosis**

a. *Physiologic lymphocytosis* occurs in healthy animals in association with fear or excitement (eg. During a conscious venipuncture). The absolute count may be markedly increased.

b. *Chronic infection* can cause lymphocytosis due to antigenic stimulation of T-cells

c. *Lymphoid leukaemia* is associated with the presence of circulating neoplastic lymphocytes – NOT necessarily associated with lymphocytosis. In some cases however, the lymphocyte count is markedly elevated and lymphocyte morphology is abnormal/ immature "blast" forms.

2. Lymphopenia

a. *Corticosteroid-induced lymphopenia* (part of the stress leucogram), but is also common in animals with debilitating diseases or in hyperadrenocorticism. The lymphopenia is partly due to destruction of lymphocytes (especially T-lymphocytes) in lymph nodes and partly to redistribution of recirculating lymphocytes into lymph nodes. Lymphopenia is observed with 4-6 hours of corticosteroid administration.

b. *Acute systemic viral infections* may cause lymphopenia.

c. *Reduced lymphopoiesis* eg. Following immunosuppressive therapy or irrsdiation.

d. *Loss, sequestration or blockage of flow of lymphocyte rich lymph* (eg. Protein-losing enteropathy)

EVALUATION OF LEUCOCYTES.

In healthy animals leucocyte morphology and numbers are relatively constant, but alterations often occur in disease. These changes are seldom specific but can provide valuable information regarding the nature of the disease process, and the prognosis. Leucocytes are routinely evaluated by estimation of total and differential leucocyte counts, calculation of absolute numbers of each leucocyte type, and examination of their morphology in blood smears (collectively referred to as the leucogram). It should be noted that a leucogram may represent a single stage of a dynamic disease process and reliable interpretation may not be possible without repeated samplings over a period of several days.

White Blood Cell Count

There is an inherent error in the haemocytometer manual dilution method of cell counts (see Part 2) –un to 20%. Hence a REQUIREMENT to study the smears as well. If nucleated red blood cells (nRBC) are detected in peripheral blood smears then the number per 100 leucocytes should be counted and the WBC count corrected by the following formula:

$$\textit{Corrected WBC count} = \textit{Initial WBC count} \times \frac{100}{100 + \textit{nRBC/100WBC}}$$

Problems can also occur if there are clumped platelets, perhaps following a difficult sampling collection.

Leucocytosis (increased WBC count) is most frequently caused by a neutrophilia rather than by eosinophilia, lymphocytosis or monocytosis. Similarly a leucopenia is usually caused by a neutropenia.

Differential Leucocyte Count

Differential leucocyte counts are obtained by examination of stained blood smears until 100 or 200 WBC's have been identified and classified. Leucocytes are usually reported as percentages as well as an absolute number of each type (% x WBC count). Interpretation of the differential count should be based on the ABSOLUTE number of each cell type rather than the percentage.

Leucocyte morphology should also be assessed in blood smears and any abnormalities noted.

THROMBOCYTES(Platelets)

Platelets play an essential role in haemostasis. Their average lifespan in circulation is 8-12 days.

1. **Thrombycytosis** This is rare, but reactive thrombocytosis may occur in association with malignant neoplasia, inflammation, haemolytic disease, iron deficiency anaemia and following splenectomy.
2. **Thrompbcytopenia** – either due to decreased production or increased destruction. Significant bleeding (petechial and echymotic) will occur if blood platelet numbers fall below 20 x 10^9/L (normally 200-400 x 10^9/L). Increased platelet consumption will occur in DIC. Note also that MEASLES causes increased destruction, and production of enlarged platelets.

Platelet evaluation

This can be done on the blood smear, preferably within 4-5 hours of smear preparation. Note however, that this can be very unreliable if there is platelet clumping at the feathered edge of the smear. Interpretive guidelines are as follows:

6 or 7 per oil immersion field = approximately 100 x 10^9/L
< 3-4 platelets per oil immersion field = thrombocytopenia
< 1 platelet/ 50 RBC is suggestive of thrombpcytopenia – but also consider anaemia

In vitro bleeding time measures the ability of platelets to form a haemostatic plug, but the test is insensitive. Its main advantage is its simplicity. Prolonged bleeding time occurs in thrombocytopenia or functional platelet defects, but NOT in deficiencies of clotting factors.

PLASMA PROTEINS

There are two main groups of plasma proteins, albumin and globulins, both of which are normally maintained within narrow limits in healthy animals.

Albumin is manufactured by hepatocytes in the liver and released into the plasma where it plays a major part in maintaining colloid osmotic preseeure. It also serves as a transport protein by binding many other substances, including bilirubin, hormones, drugs, and approximately 50% of the total serum calcium. Ambumin has a low molecular weight (69 000) but in normal animals it is NOT excreted in the urine. Estimates of its half life vary from 8 to 23 days.

The **globulin** fraction consists of a diverse group of proteins with a broad range of functions. They can be separated by electrophoresis into three main groups: alpha, beta and gamma.

Alpha globulins are produced by the liver and are primarily either transport proteins or enzymes (eg. Hapto globin, various glycoproteins.

Many beta globulins also originate in the liver, including beatlipoproteins and complement. The immunoglobulins IgA and IgN are also beta globulins.

Gamma globulins are synthesised by B-lymphocytes (or plasma cells) and are primarily IgG antibodies.

ALTERATIONS IN PLASMA PROTEIN CONCENTRATIONS

The detection of abnormalities in the concentration of total or specific plasma proteins can provide valuable information on the nature of a disease process.

1. Total Protein

Total plasma protein concentration is often measured by refractometer as part of a routine haemogram to provide an indication of the state of hydration. An elevated total protein concentration is most often secondary to dehydration, although increased globulin concentrations in animals with chronic infections or multiple myeloma will occasionally cause significant elevations in total protein. In such cases electrophoresis is required to identify which globulin fractions are increased.

Whenever the total plasma protein concentration is reduced it is important to determine whether the reduction is due to a panhypoproteinaemia (lowered albumin and globulin fractions) or to a reduction in just one fraction. Panhypoproteinaemia is a feature of such conditions as blood loss, protein-losing enteropathies, maldigestion/ malabsorption syndromes and MALNUTRITION.

2. Albumin

Hyperalbuminaemia is almost invariably associated with dehydration but artifactual increased albumin can occur in haemolysed or lipaemic samples. **Hypoalbuminaemia** can have many causes as indicated in the following:

a. Reduced synthesis
   i. Chronic liver disease
   ii. Malnutrition
   iii. Intestinal malabsorption
   iv. Exocrine pancreatic insufficiency
b. Accelerated loss
   i. Haemorrhage
   ii. Renal disease (proteinuria)
   iii. Protein-losing enteropathies (eg. Parasitism)
   iv. Severe exudative skin disease
   v. Protein-rich effusions

In most of these conditions hypoalbuminaemia will be accompanied by hypoglobulinaemia and the A/G ratio will be normal. A reduced A/G ratio will occur in chronic liver disease and renal disease where there is selective reduction in albumin. The liver has a large functional reserve and significant reduction of albumin synthesis will not occur until 60-80% of the liver has been destroyed.

When plasma albumin concentrations fall below a threshold level of around 10-12g/L the colloid osmotic pressure within the blood is so loa that fluid is lost into tissues or body cavities, resulting in ascites, hydrothorax and pulmonary oedema.

3. **Globulins.**

Hyperglobulinaemia may be due to dehydration or to an increased concentration of one of the fractions. Polyclonal gammopathies tend to be assicated with certain chronic inflammatory diseases, while monoclonal gammopathies often indicate neoplasia (excessive production of a single class of antibodies by neoplastic plasma cells). This differentiation can only be made using protein electrophoresis.

Hypoglobulinaemia is most often seen together with hypoalbuminaemia, due to the causes noted above.

PART 2. The Haemogram.

This section is designed as a step by step guide on performing an accurate haemogram. The whole process shouldn't take longer than 15-20 minutes, and can provide a lot of important information. At the end of the section there is a series of colour slides both on normal and abnormal cells, as well as current ISIS data on primate blood normals. For more indepth information, please refer to Part 1.

BLOOD SMEAR PREPARATION

Cap tubes - red topped are Hepa

Examination of a well-prepared, well-stained blood smear is potentially one of the most valuable laboratory tests. Although the staining and interpretation of blood smears will often be done by a diagnostic laboratory the smears should be made at the time of blood collection (i.e. by the veterinarian or an assistant).

Ideally, smears should be made from fresh blood not containing an anticoagulant. Good quality smears can be prepared from whole blood containing EDTA anticoagulant, but they should be made as soon as possible after collection (within 1 hour).

The technique most commonly used for preparation of blood smears is illustrated in Fig. 1.

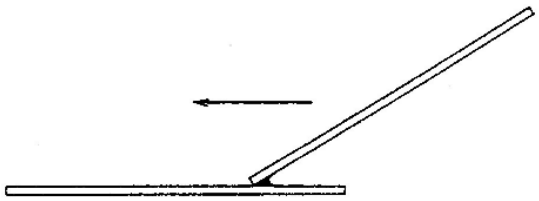


Fig. 1. Slide method.

A small drop of blood is placed near one end of a clean slide (free of dust or fingerprints). A second slide (spreader slide) is immediately placed against the surface of the first slide at an angle of about 30° and drawn back into the drop of blood. When the blood has spread along at least two-thirds of its width the spreader slide is pushed forward with a steady, even motion. The blood will follow making a film which is thickest at the start and tails off into a thin "feathered" edge near the end of the slide. If the smear runs off the end of the slide then the drop of blood was too large and another smear should be prepared.

Dry the smear rapidly by waving it in the air or in front of a fan, but do not use heat. Slow drying enhances creation of erythrocytes due to movement of fluid out of cells into the plasma.

Staining of air-dried smears can be delayed for several weeks if necessary, but they must be kept clean and dry.

Staining Blood Smears

Most stains used routinely for staining blood smears are modified Romanowsky stains based on a combination of methyelene blue and eosin. Acidic dyes such as eosin combine with basic components of the cell (e.g. cytoplasm) producing a pink, red or orange colour. Methylene blue is a basic dye and stains acidic parts of the cell (e.g. nucleic acids) blue, purple or violet.

The following commonly used stains: Wright's, Leishman's, MacNeal, Giemsa and May-Grunwald are all modified Romanowsky stains. It is not necessary to have all these available in a veterinary practice laboratory. Excellent results can be achieved with the commercial quick-dip stains such as Diff-Quik, which can also be useful for staining cytological preparations.

A well stained blood smear will appear pink or mauve to the naked eye. Microscopically the red cells will be pink, eosinophil granules red and leucocyte nuclei purple/ blue.

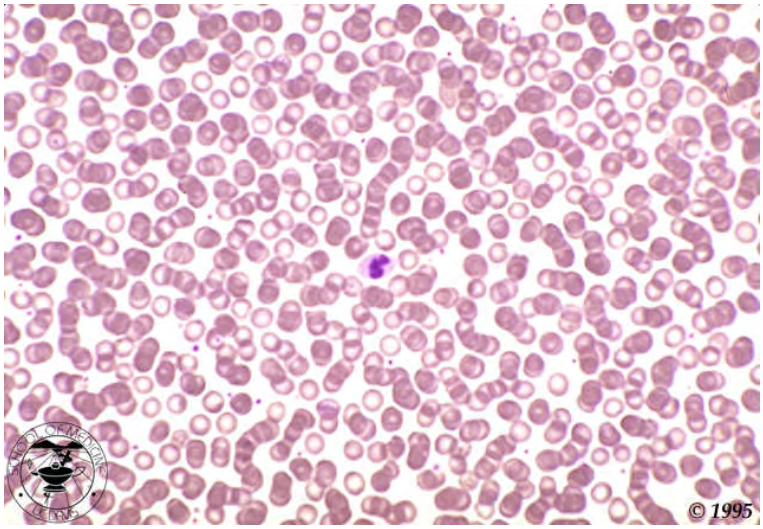


Figure This smear is ***TOO THICK***

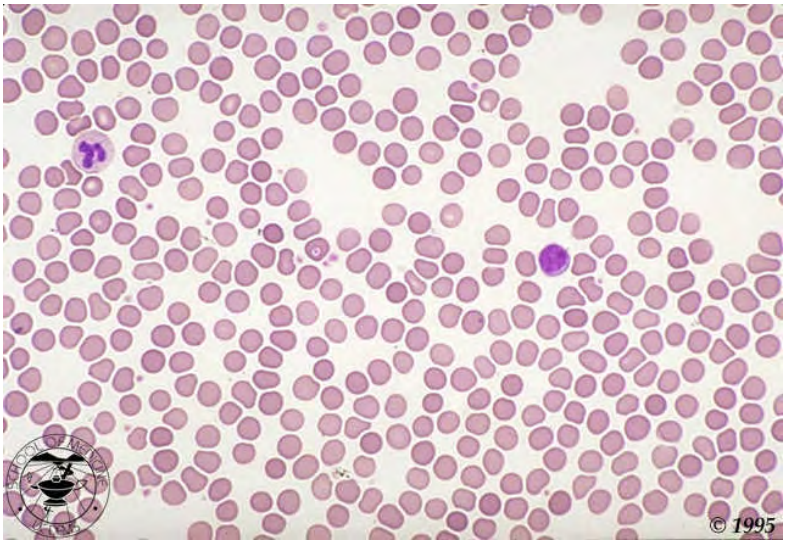


Figure This smear is marginally **TOO THIN**

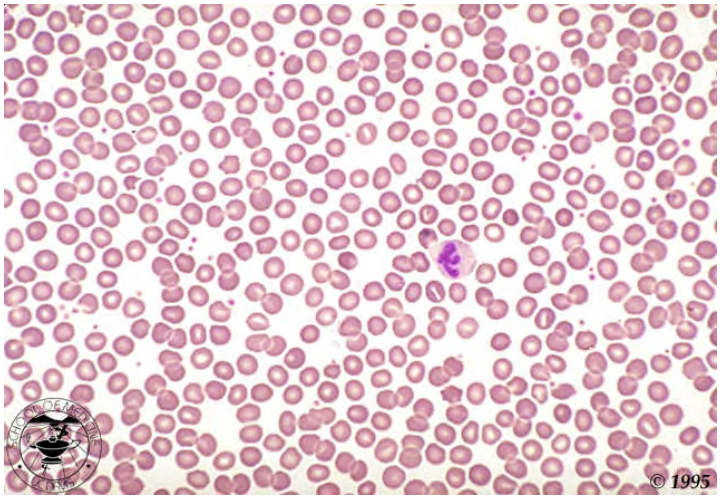


Figure This is a **GOOD SMEAR**.

HAEMOGRAM/CBC

The haemogram or complete blood count (CBC) is probably the most common laboratory examination performed on sick animals, and can provide valuable information on the nature of a disease process.

1. Packed Cell Volume (PCV)
The simplest and most accurate method for estimating the PCV is the **microhaematocrit method** which requires a high spped microhaematocrit centrifuge and capillary tubes (75mm x 1mm). Plain capillary tubes (blue tip) are used with blood containing anticoagulant while heperanised tubes (red tip) are available for use with fresh blood.

METHOD:

- Place the clear end of the capillary tube into a drop of fresh blood or well mixed unclotted blood and allow it to fill (2/3 – ¾) by capillary action
- Seal the colour coded end with plasticine
- Centrifuge for 5 min at 10-13000rpm (with sealed end outward!!)
- After centrifugation measure the PCV using a reader card or micro-capillary reader.

The microhaematocrit method is rapid, accurate (± 1%) and requires only 3-4 drops of blood.
Red discolouration of the plasma indicates haemolysis and although in most cases this will have occurred during or after collection of the sample it may be a significant finding in an animal with anaemia and/or a positive blood test on the urine. Yellow discolouration of the plasma usually indicates hyperbilirubinaemia in primates. White or pink, opaque plasma ('tomato soup') reflects lipaemia and is caused by a high concentration of chylomicrons. The thickness of the buffy coat is also worth noting as, with ecperience, it can provide a useful approximation of the total leucocyte count.

TIP: If the erythrocytes are of normal size (determined from a blood smear examination) then an approximation of the haemoglobin concentration can be obtained by dividing the PCV by 3.

INTERPRETATION OF PCV
PCV is a measure of the concentration of RBC's in circulation and although a reduced value usually indicates anaemia it could also be the result of overhydration (eg. Following excessive infusion of parenteral fluids). An increased PCV indicates polycythaemia but is most often a result of either dehydration or excitement during blood collection (with splenic contraction) rather than a true increase in the body's total red cell mass.

Remember, normal ranges vary between species (see ISIS reference ranges in this manual). Newborn animals usually have a PCV within the normal adult range (or even higher) but this declines soon after birth and remains low for the first few months of life.

Erythrocyte counts, from diluting pipettes and a haemocytometer, are not very accurate and are only likely to be of value in anaemic animals where calculation of the mean corpuscular volume (MCV) or absolute numbers of reticulocytes may provide useful information.

2. Reticulocyte Count.
A reticulocyte count should always be done if the PCV is low as it can help to indicate whether or not the anaemia is regenerative.

Reticulocytes are stained supra vitally by incubation with a dye such as brilliant cresyl blue, new methylene blue (NMB) or modified NMB. Chains or clusters of residual RNA in reticulocytes are stained dark blue by these dyes and produce a characteristic reticular pattern.

Heinz bodies and Howell-Jolly bodies will also be stained by these dyes.

METHOD:

- Mix equal amounts of blood and stain (2-3 drops of each) in a small test tube.
- Incubate at room temperature for 15-20 minutes
- Prepare a blood smear from the mixture and count the number of reticulocytes per 500 erythrocytes. Express the result as a percentage. NMB stain is not permanent and smears should not be left for more than 1-2 days before examination

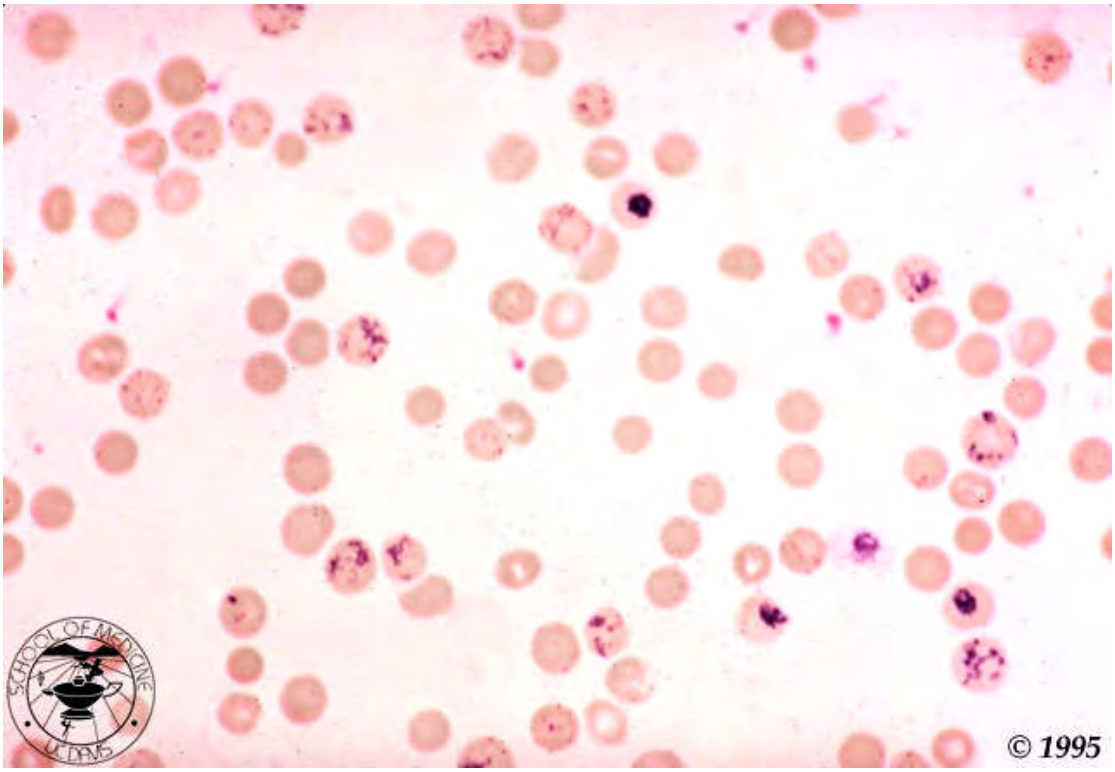


Figure Blood smear indicating reticulocytosis

3. Total White Blood Cell Count.
Estimated white cell count from smear:
This is a reliable, simple method for obtaining an estimate of the total number of WBC's. Although it is only an estimate, it is the method preferred by some clinicians because they find it simple, convenient (no time limitations) and consistently reliable. The count is performed on a stained blood smear. For optimal results, it is essential to have a well-made and well-stained smear with a good monolayer and even distribution of cells.

The procedure is a s follows:

- Scan the stained smear on low power (10x objective) to check the distribution of cells and locate an area where there is a good monolayer.
- Examine the selected area with a 40x objective. Count all the WBC's in TEN (10) evenly distributed fields. DO NOT use fields with clumped WBC's or large numbers of smudge cells because they will deleteriously affect the estimate
- Divide the toal number of WBC's counted in the 10 fileds by 10, thus determining the average per field. Multiply this by 1.6 to give the number of cells x 10^9/L.

Eg. Total number of WBC's counted in 10 fields = 150.
Divided by 10 = 15
Multiply by 1.6 = 24
Therefore estimated total WBC count is 24 x 10^9/L

The count should be expressed as a RANGE, following the guidelines below:

WBC x 10^9/L	Range Spread
<25	2
25-40	4
40-65	5
65-140	10
>140	20

Table 9

Therefore in the example above, the range would be 23-25 x 10^9/L

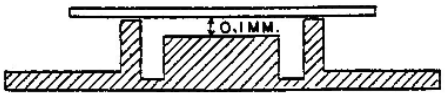
- This method is based on the ratio of WBC's tp RBC's in the blood. Therefore, if the PCV is outside the normal range for most primates (say 38-55%), then the estimated total WBC count should be corrected using the following formula:

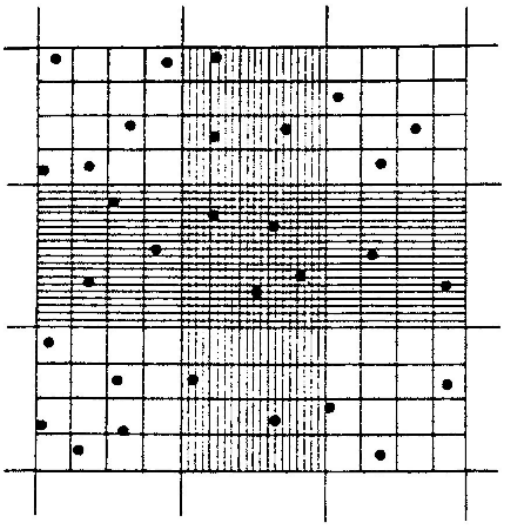
Corrected WBC count = estimated WBC count x $\frac{\text{observed PCV}}{43.8}$

(where 43.8 is the average PCV for the chimpanzee)

For example – If in the above case the chimp had a PCV of 34, the estimated WBC count would be corrected as follows:
Corrected WBC count = 30x10^9/L x $\frac{34}{43.8}$ = 23 (range 22-24x10^9/L

The total WBC count is a valuable component of a haemogram, and can be easily done using a Neubauer haemocytometer. The haemocytometer has a counting chamber 0.1mm deep with a ruled are as illustrated below:





Hemacytometer grid. Using Unopette test #5853, the observer counts the leukocytes in the total grid area. Only one of the nine major squares is completely in view at one time under the 10× objective. The field must be moved from one major square to the next while proceeding through the count.

The four large corner squares, each of which are 1mm square, and are used for leucocyte counts in the Thoma pipette method, but all 9 large squares are counted in the "Unopette" method. The coverglass and the ruled area of the haemocytometer must be kept free of grease and dust. The coverglass is placed on the supporting ribs of the counting chamber (handling only the long edges). The chamber is filled by capillary action when the diluting pipette or capillary tube is touched to the space between the coverglass and the haemocytometer. If the chamber is overfilled and fluid runs into the trough around the counting platform the haemocytometer should be raised, dried and reloaded.

After loading, the haemocytometer is allowed to settle in a moist chamber for approximately 5 minutes (eg. In a Petri dish with moist filter paper).

Leucocytes are counted using a systemic approach which avoids duplication.

Nucleated erythrocytes will be counted along with the leucocytes, and if seen to be present in subsequent examination of a blood smear the leucocyte count must be corrected by the following formula:

Corrected WBC count =

$$\textit{Initial WBC count} \times \frac{100}{100 + nRBC/100\ WBC}$$

Unopette Method:
The "unopette" method for WBC counts is ideal, and can also be used for platelet counts. Blood is collected into a capillary pipette (which holds 20uL) and mixed with 1.98mL of AMMONIUM OXALATE diluent in a reservoir

giving a final dilution of 1:100. The diluent lyses erythrocytes but not WBC's or platelets.

WBC's in all 9 large squares of the haemocytometer are counted under 100x magnification. The number of cells divided by 9 gives the total WBC count ($x10^9/L$).

4. Evaluation of Blood Smears.

This is probably the most important single component of the haemogram and, with experience, can yield a range of valuable information. But in order to get the maximum benefit from a blood smear, it must be prepared within an hour or so of collection, and must be well stained.

A systematic approach for examining blood smears should be developed so that nothing will be overlooked. An initial scan on low power (4x or 10x) will give an overall impression of the quality of the smear and stain, the degree of variation in erythrocyte size and shape, the distribution of leucocytes and an approximation of leucocyte and thrombocyte numbers. This is followed by more detailed examination of the different cell types, usually with either a 40x or 100x immersion lens.

(a) Erythrocytes

Inspection of erythrocytes should include consideration of each of the following:

- **Size –** variation (anisocytosis), normocytic, macrocytic or microcytic. Remember there is species variation in RBC size, but primates should all be fairly similar (see photos). Macrocytic RBC's are seen in Vit B12 or folate deficiencies (megaloblastic anaemias). Microcytic (smaller than normal) RBC's are often seen in severe burns, where they have burst due to high temperatures.
- **Shape** - variation (poikilocytosis), spherocytes, target cells, acanthocytes, fragmented RBC's.
- **Colour –** the staining intensity can give an indication of whether the RBC's are normochromic or hypochromic.
- **Abnormal conditions –** eg. Polychromasia (indicates a regenerative anaemia), basophilic stippling, presence of nucleated RBC's, parasites
- **Inclusions –** eg. Howell-Jolly bodies, Heinz bodies, viral inclusions, parasites.

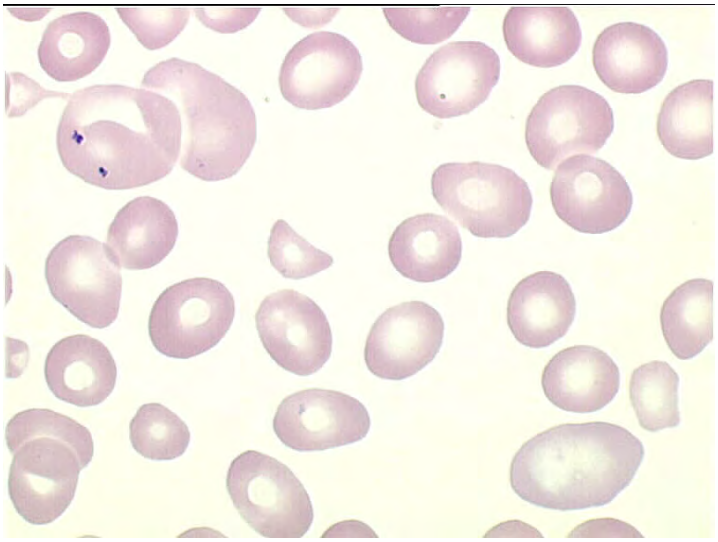


Figure Smear indicating **macrocytes**

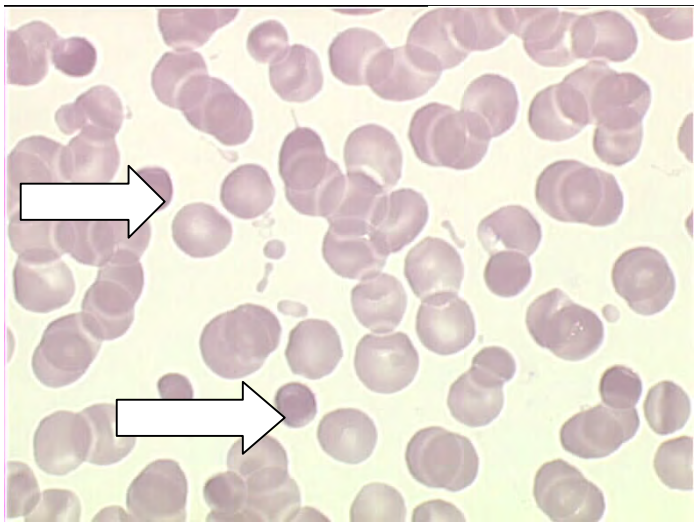


Figure Smear indicating **microcytes**

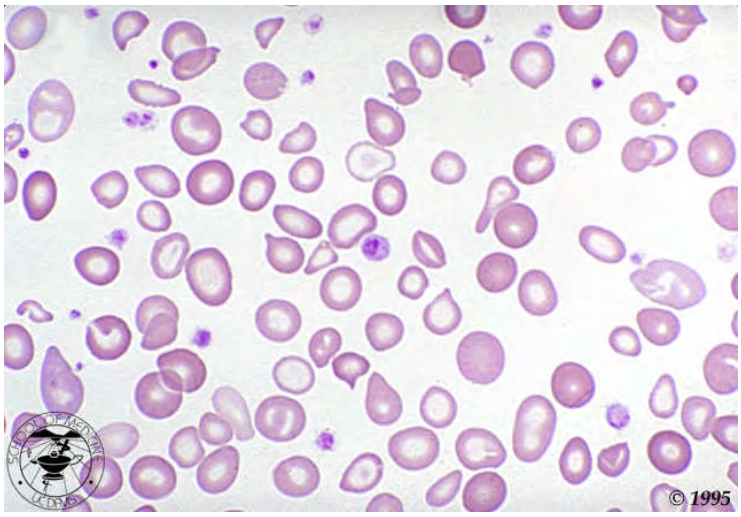


Figure Microcytic anaemia (iron deficiency)

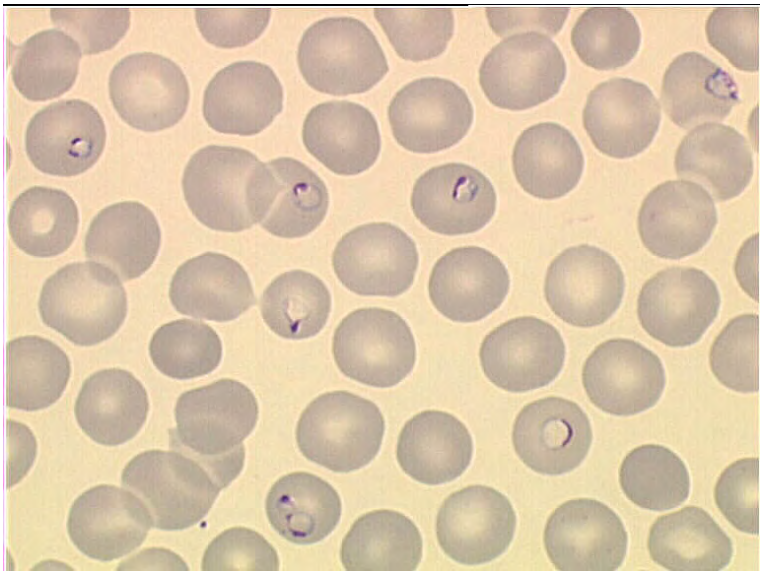


Figure Plasmodia in RBC's

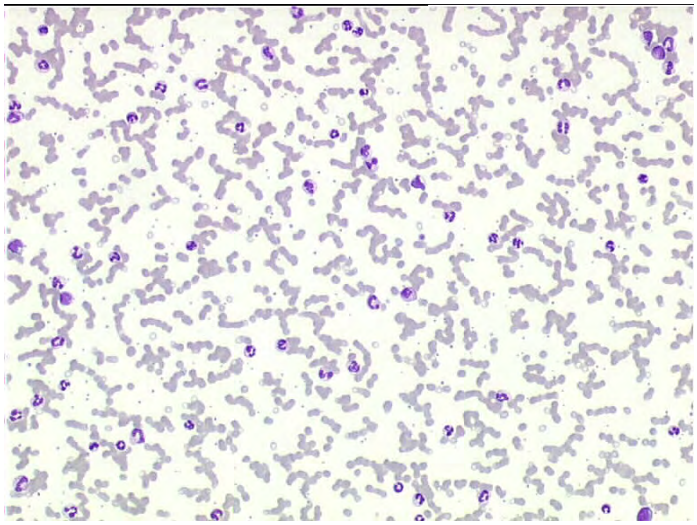


Figure EXCESSIVE linear aggregation of erythrocytes (rouleaux formation) may indicate a toxaemia, but is often seen in inflammatory disorders.

Alterations in erythrocyte morphology or the presence of abnormal inclusions can provide useful clues as to the cause of an anaemia but must be differentiated from artefactual and incidental findings. Some of the more common alterations are listed below:

Artifact: Should always be considered in a clinically normal animal that presents an abnormal blood smear. The example below indicates water contamination of the smears, rather than a disease process.

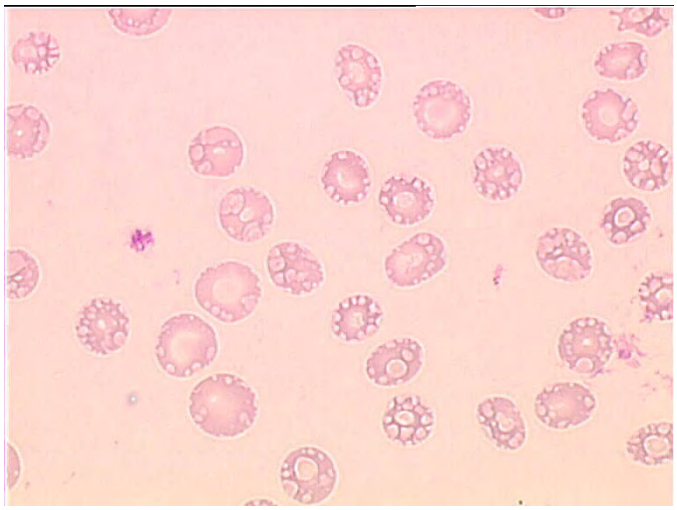
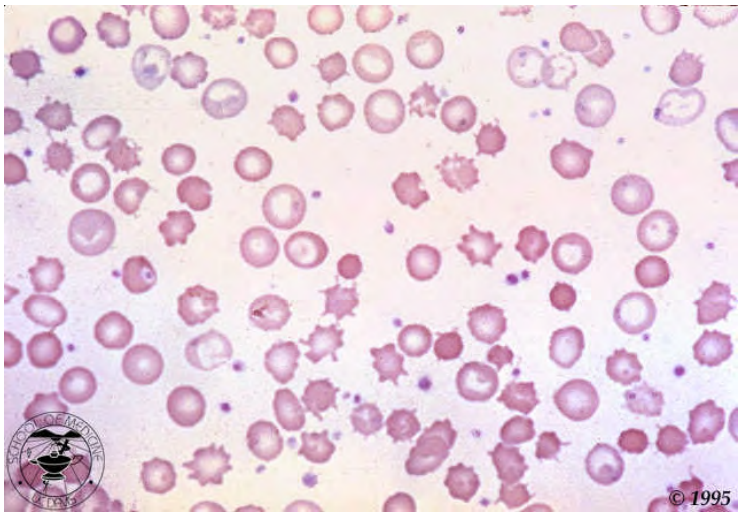
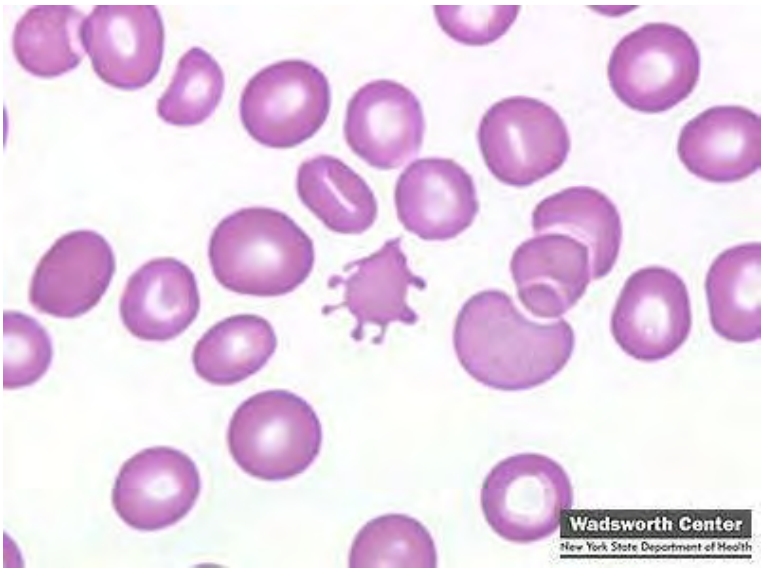


Figure Water contamination artifact

Acanthocyte (spur cell) – cells with a few to many blunt surface projections of irregular length. They are smaller than crenated cells and have fewer projections. These are RARE, but are seen in a variety of liver diseases and possibly portocaval shunts.





Figue (a and b) Anacthocytes (Spur cells)

Basophilic stippling – erythrocytes with scattered basophilic granules. (indicated with arrows in the pictures below). This can be normal in the newborn, but is usually related to abnormal haemoglobin synthesis in the older animal. The particles are precipitated ribosomal protein (RNA) seen in lead poisoning, severe bacterial infection, drug exposure, alcoholism, anemias with impaired hemoglobin synthesis, megaloblastic anemia, and refractory anemia.

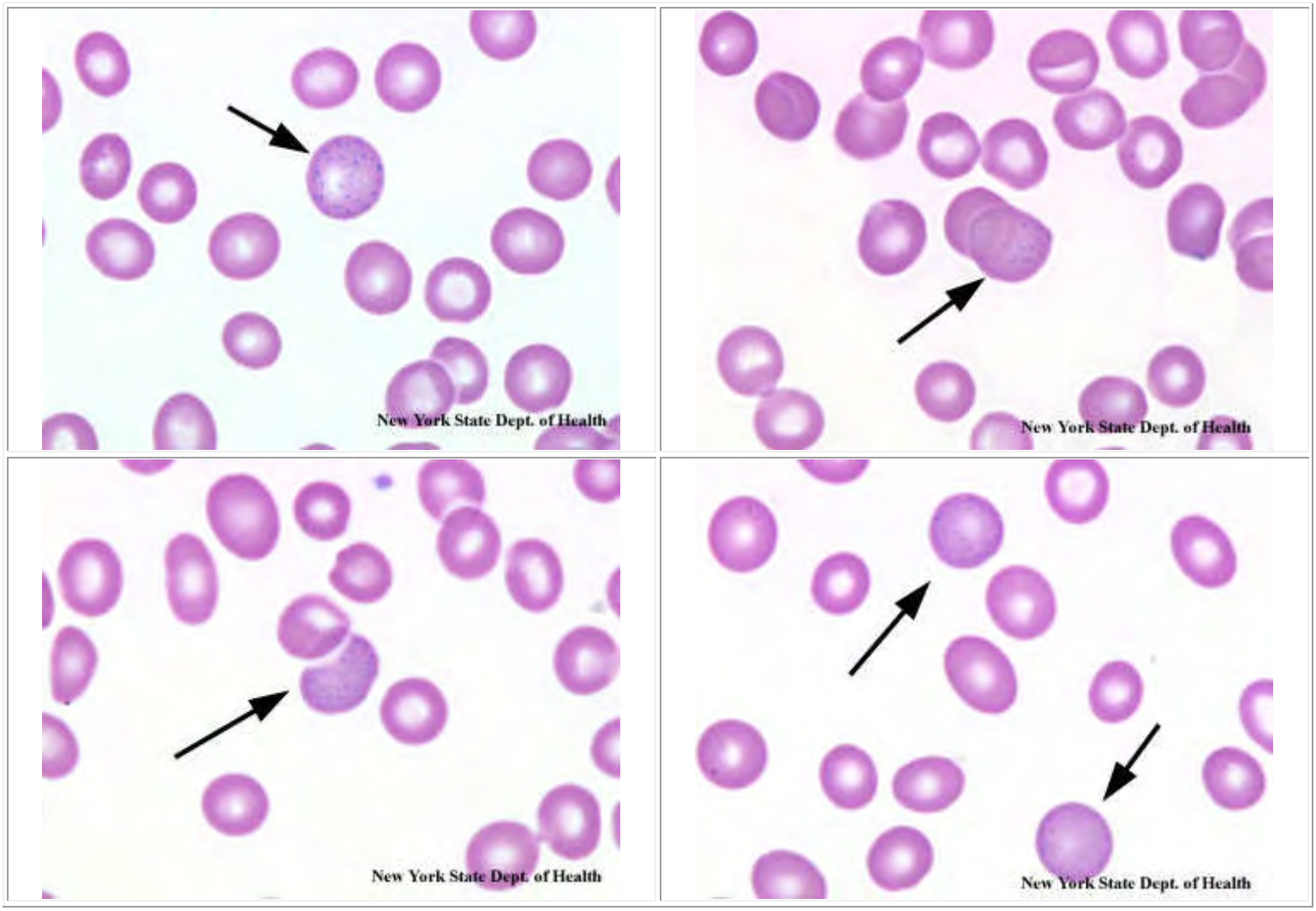


Figure Basophilic stippling

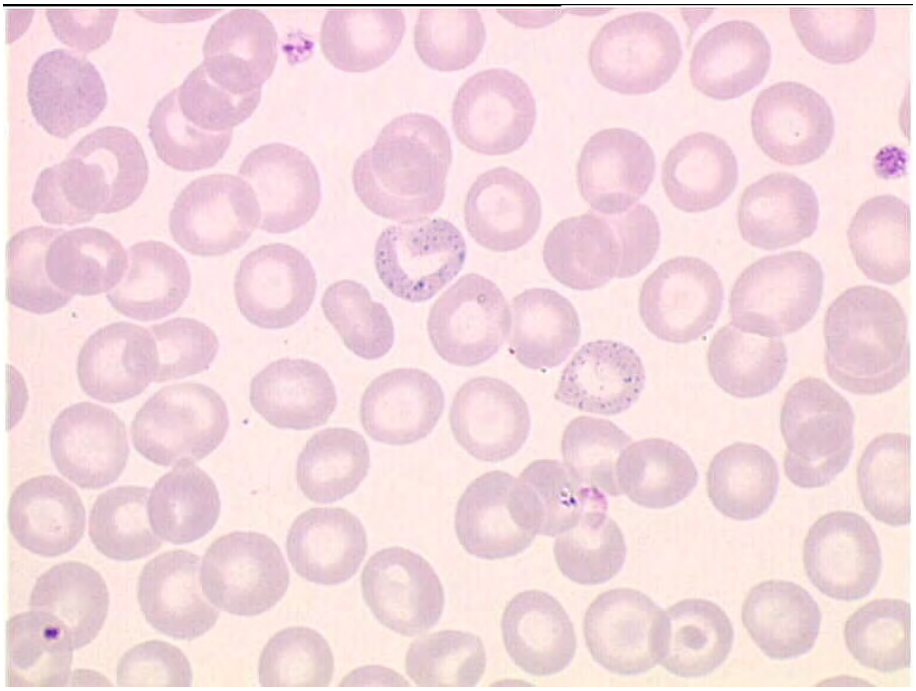


Figure Basophilic stippling

Crenated Cells – also called **echinocytes** or **burr cells**. These have 10-30 short, blunt, evenly spaced surface projections and are frequently the result of shrinkage due to improper preservation or smearing of the blood – ie, an artefact. Occasionally it may be due to uraemia (excess urea in the blood), or acute renal failure.

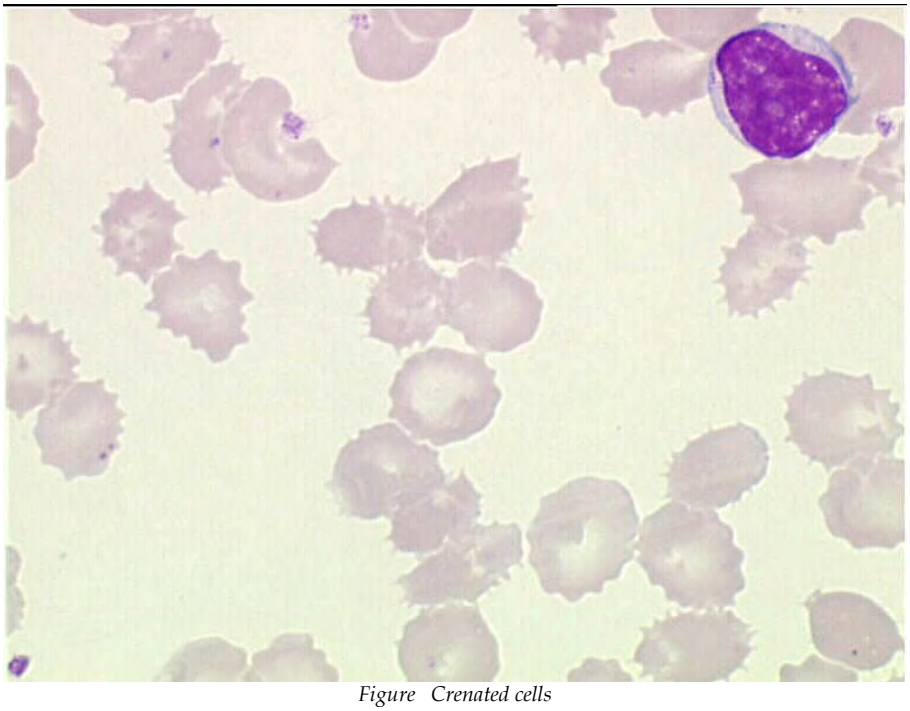


Figure Crenated cells

Heinz bodies – clumps of denatured haemoglobin on the internal surface of the erythrocyte membrane. They appear as unstained retractile blebs on Wright's (Diff Quick) stain, but are blue on supra vital stains such as new methylene blue. Heinz bodies reflect oxidative damage to haemoglobin by

certain chemicals and drugs (eg. Acetaminophen, phenothiazine, primaquin) and in certain types of hereditary haemolytic anaemia.

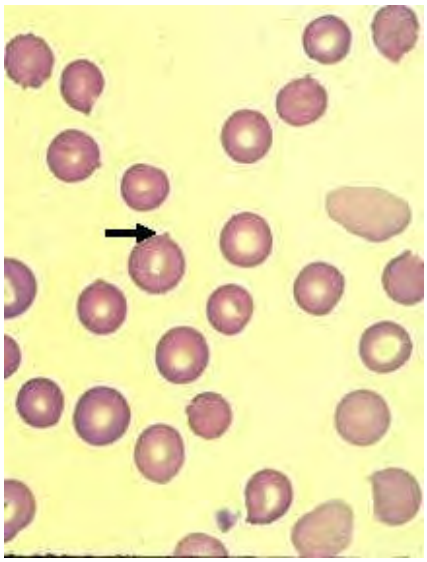
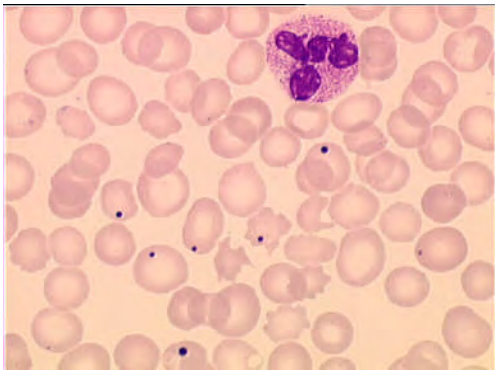


Figure Heinz Bodies. Wright's Stain, 500x

Howell-Jolly bodies – these are small, densely staining, spherical bodies within erythrocytes and are considered to be nuclear remnants. They will occur normally in small numbers. They usually occur in excess with regenerative anaemia, but may also be associated with chronic steroid therapy. Normally, RBC's that contain one HJ body are eliminated from the blood stream by the spleen within a few minutes.



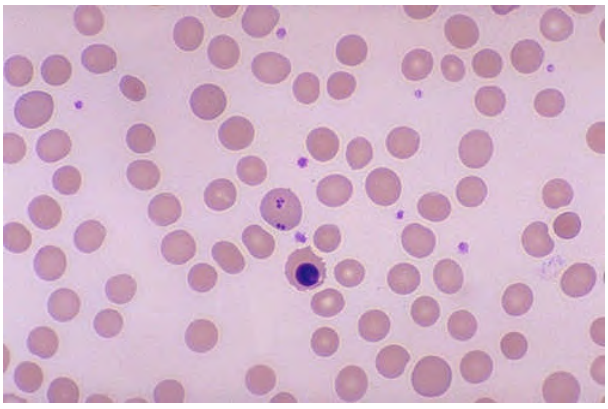


Figure (a and b) Howell-Jolly bodies

Hypochromasia – poor staining of erythrocytes. Seen in iron deficiency anaemia

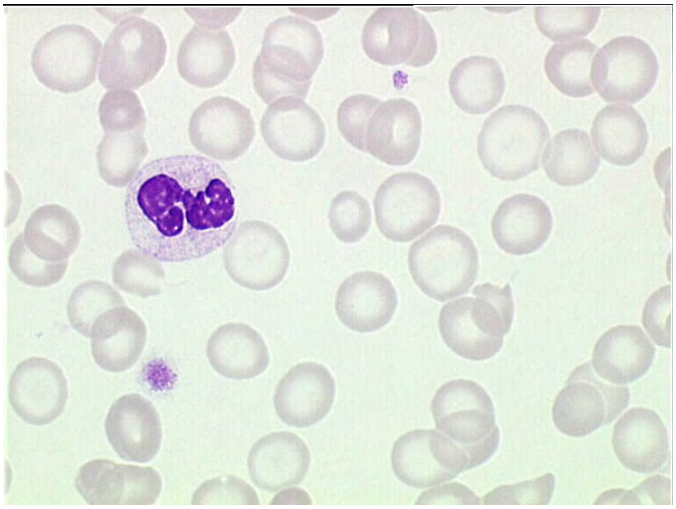


Figure Hypochromasia

Leptocytes (codocytes, target cells) – thin, cup shaped erythrocytes with a surface area too large for their volume. They fold or flatten into pale target cells with a central smudge of haemoglobin. Leptocytes may be the result of either excess cell membrane (eg. In obstructive hepatic disease) or reduced haemoglobin content (eg. Iron deficiency anaemia).

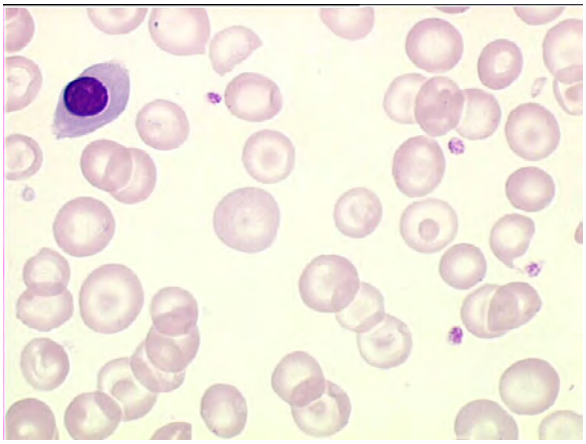


Figure Leptocytes

Metarubricytes (nucleated erythrocytes) – usually reflect the release of immature cells during anaemia but may be due to bone marrow damage.

Poickilocyte – abnormally shaped erythrocyte. Poikilocytosis is used as a general term embracing all changes in the shape of the erythrocytes, rather than a specific alteration. It usually indicates a problem with RBC production.

Polychromasia – variation in colour (staining affinity) of erythrocytes, caused by the presence of the more basophilic reticulocytes in addition to normal mature erythrocytes – ie, indicates a regenerative response in anaemia. Polychromasia is normal in the newborn.

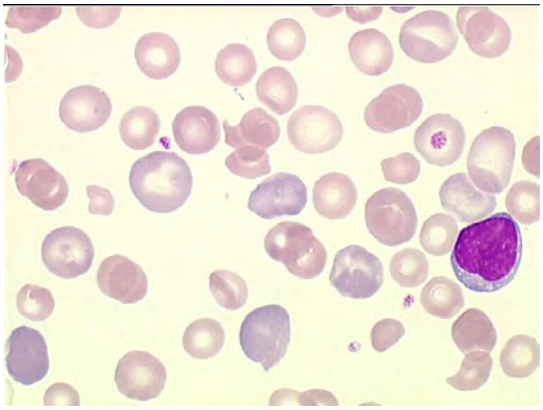


Figure Polychromasia

Reticulocyte – large, non nucleated erythrocyte containing sufficient RNA to stain as granules or a network of delicate fibrils with supra vital stains such as NMB (indicated by the arrows in the picture below). Reticulocytes are the hallmark of regenerative anaemia in most species. Because of the large size of reticulocytes, anisocytosis is a feature of blood smears from animals with reticulocytosis.

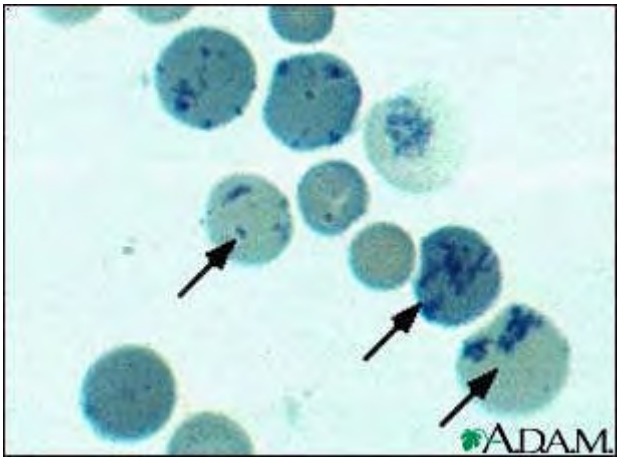


Figure Reticulocytosis

Schistocytes (schizocytes) – erythrocyte fragments with sharp edges. Caused by collisions between erythrocytes and fibrin strands (eg. Mechanical obstacles such as the heart valves), in animals with DIC and microangiopathic haemolytic anaemia and in haemangiosarcomas. Shistocytes may also occure in association with iron deficiency, uraemic syndromes, glomerulonephritis, heart failure and burns. Schistocytes can swell to become Spherocytes.

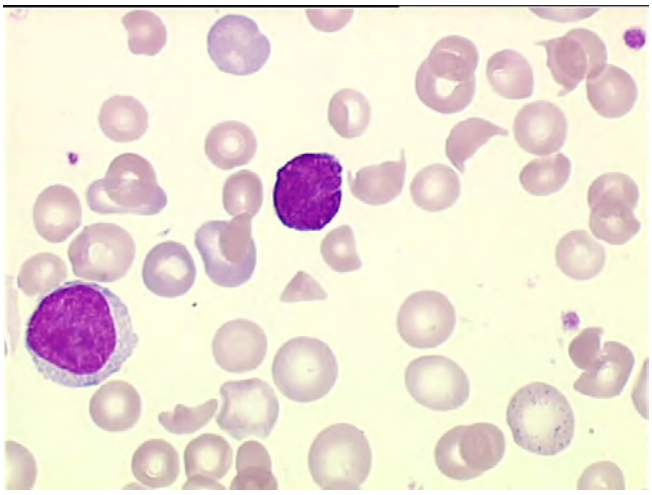


Figure Schistocytes

Spherocytes – small, dark, spherical erythrocytes which result from partial phagocytosis (removal of membrane fragments) by the tissue macrophage system. The presence of spherocytes strongly suggests a diagnosis of immune mediated haemolytic anaemia. The second picture below also shows polychromasia.

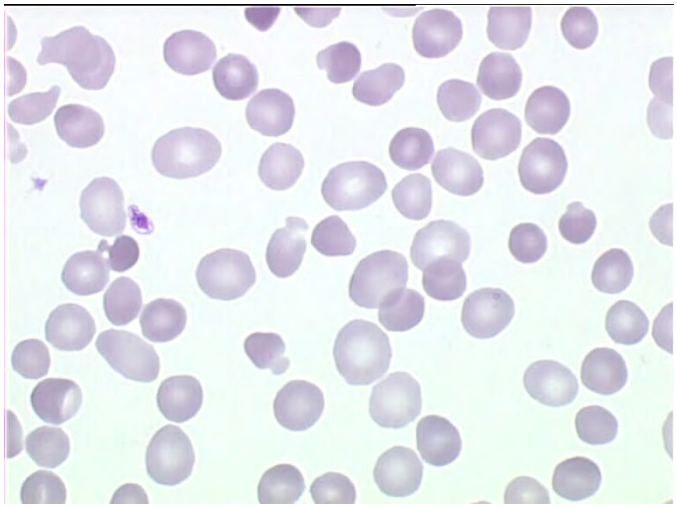


Figure Spherocytes

(b) Leucocytes.

Blood smears are required for **differential leucocyte counts**. At least 100 leucocytes are counted and classified, but if the total WBC count is significantly elevated or if the proportions of different cell types are altered then the count should include 200 cells for greater accuracy.
Even in well-made smears the distribution of the different types of WBC is not uniform. Neutrophils tend to accumulate along the margins of the smear, lymphocytes are most common a short distance from the margin, while monocytes and eosinophils are scattered throughout. In order to overcome this problem a "battlement" pattern for counting leucocytes is recommended:

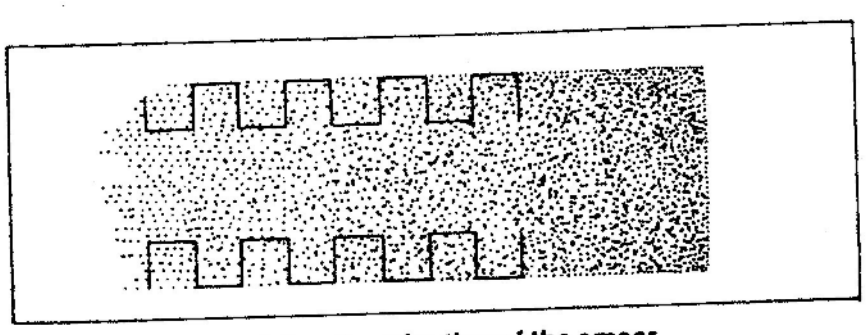


Fig. 3.3. Examination of the smear.

Percentage values of each type of cell are of LIMITED diagnostic value and MUST be converted to absolute numbers for interpretation. A total WBC count is therefore necessary (see previous section). Some causes of alterations in leucocyte numbers are listed in the following table:

	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Increased by	Infection	Excitement	Stress	Allergy
	Inflammation leukaemia	Lymphocytic leukaemia		Parasitism
	Toxaemia (eg. Uraemia)	Adrenocortical insufficiency	Chronic inflammation	Tissue breakdown
	Neoplasia			
	Stress/ corticosteroid therapy	Some chronic infections	Monocytic leukaemia	Adrenocortical insufficiency
	Exertion			
	Myelogenous leukaemia			Leukaemia
Decreased by	Viral infection	stress		Stress
	Peracute bacterial infection	Chronic disease		Corticosteroid therapy
	Bone marrow depression/ invasion	Some viral diseases		
		Hyperadrenalism		
		Cortocosteroid therapy		

An important part of the differential count is the identification of immature neutrophils (band cells and metamyelocytes) signifying a **left shift** (severe acute (usually) bacterial infection).

Band neutrophils have a smooth U-shaped or horseshoe-shaped nucleus whith less chromatin clumping than in mature neutrophils and no segmentation. The nucleus of a band cell should not be indented by more than half of its thickness. **Metamyelocytes** may appear in peripheral blood together with band cells in animals with a severe left shift. They have a kidney-shaped nucleus and pale grey cytoplasm. Band cells and metamyelocytes can be confused with monocytes in blood smears. Monocyte nuclei are often hidney-shpaed, but the cytoplasm is usually more basophilic than that of meta myelocytes and often contains vacuoles.

It is useful to remember that metamyelocytes will only be present in circulation of there is a significant left shift (ie. Many band cells).

Leucocyte morphology should also be assessed in blood smears. One of the most common abnormalities is the presence of **toxic change** in the neutrophils suggesting a bacterial septicaemia or toxaemia. Toxic changes in neutrophils include cytoplasmic basophilia and vacuolation, Dohle bodies, toxic granulation and giant, bizarre forms probably resulting from a maturation defect.

Neoplastic leucocytes (lymphocytes, granulocytes, monocytes, mast cells) can be identified in blood smears from animals with leukaemia, NOT always in association with an increased total WBC count. Neoplastic cells are often large with deeply basophilic cytoplasm and pale staining nuclei containing one or more prominent nucleoli.

(c) Platelets

Thrombocytes appear in routine blood smears as pale purple, granular bodies, variable in size but usually much smaller than erythrocytes. They may occur singly or in clusters and an average of 10 or more per high powered field is considered to be normal. However, this does not necessarily mean that their function is normal.

An apparent deficiency of thrombocytes in a blood smear (< 3 per hpf) may be artifactual due to clumping during or soon after collection. This is especially likely to occur after difficult venipuncture where the blood sample may become contaminated with tissue thromboplastin.

If large clumps of platelets are present near the feathered edge of the smear then platelet numbers are probably normal. However, if such clumps are not present, and there are few platelets throughout the smear, thrombocytopenia should be suspected. This could be confirmed by a platelet count using a haemocytometer (by counting all the platelets within the 25 small squares within the large square in the middle – see previous illustration). Normal count is 150-800 $x10^9$/L.

5. Total Plasma Protein

Plasma protein estimations can be readily performed using a refractometer and are routinely included in haemograms. The simplest method is to use the plasma from centrifuged microhaematocrit tubes. The tube is broken immediately above the buffy coat and plasma drained onto the refractometer prism.

The estimation is accurate providing the plasma is not visibly lipaemic (cloudy) and there is no evidence of haemolysis.

Total plasma protein concentrations in adults of most species range from 60-75 g/L (73 in chimps).

Interpretation:

Elevated total protein concentrations (hyperproteinaemia) most often reflects dehydration and in such cases it is important that the PCV is interpreted with caution. Mild anaemias, for example, could be masked by dehydration and routine estimation of total protein can therefore be a valuable safeguard. Similarly, total protein concentrations can help to differentiate between polycythaemia caused by dehydration and splenic contraction.

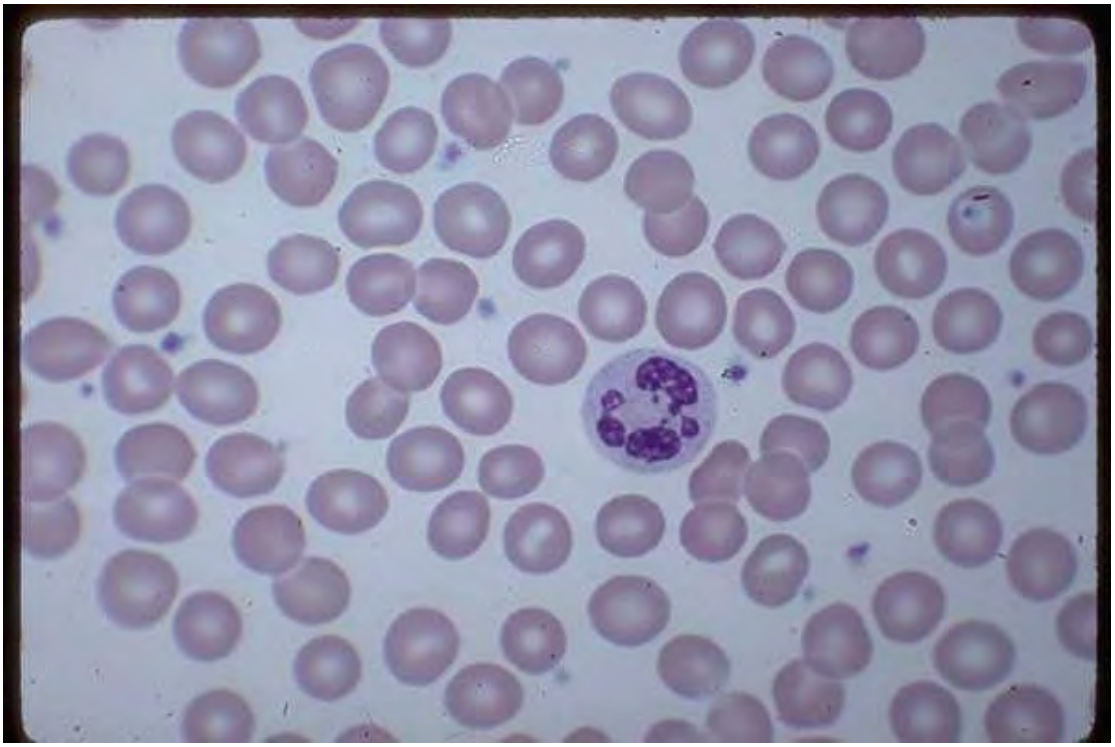
Hyperproteinaemia may also be due to hyperglobulinaemia in animals with chronic infections or plasma cell myelomas.

Low plasma protein concentrations may be due to a deficiency of albumin or globulins (or both), and can therefore have a variety of causes. In such cases, serum may need to be sent to a diagnositic laboratory (if possible) to differentiate this.

The following pictures are a series of slides obtained from various websites, and could form the basis for a growing haemogram library.

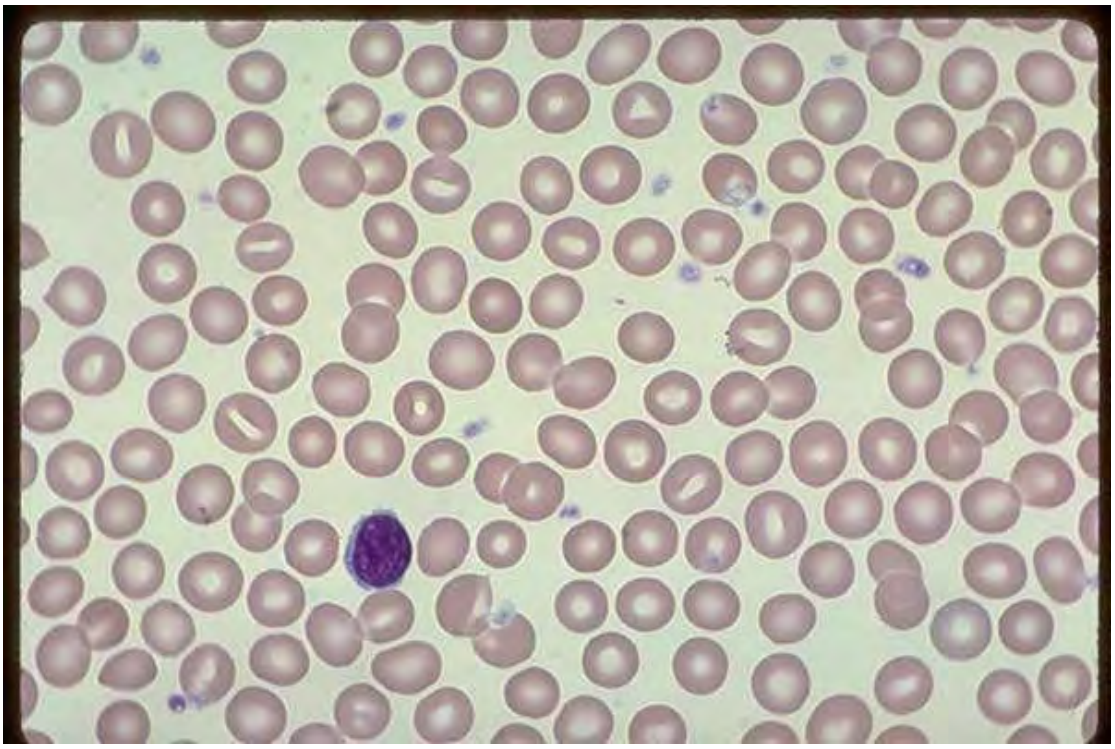
www.image.bloodline.net
Normal Platelets, Normal EDTA anticoagulated blood 1.

Normal Platelets scattered throughout the field. Note one within the halo (biconcave) area of a red cell at top right. The normal average number of platelets in a proper field ranges from 8 to 15. In EDTA anticoagulated samples they are more evenly distributed, are usually rounded up and may be slightly swollen and/or slightly degranulated. One segmented neutrophil. Normocytic, normochromic RBC. Normal EDTA anticoagulated blood - 100X objective



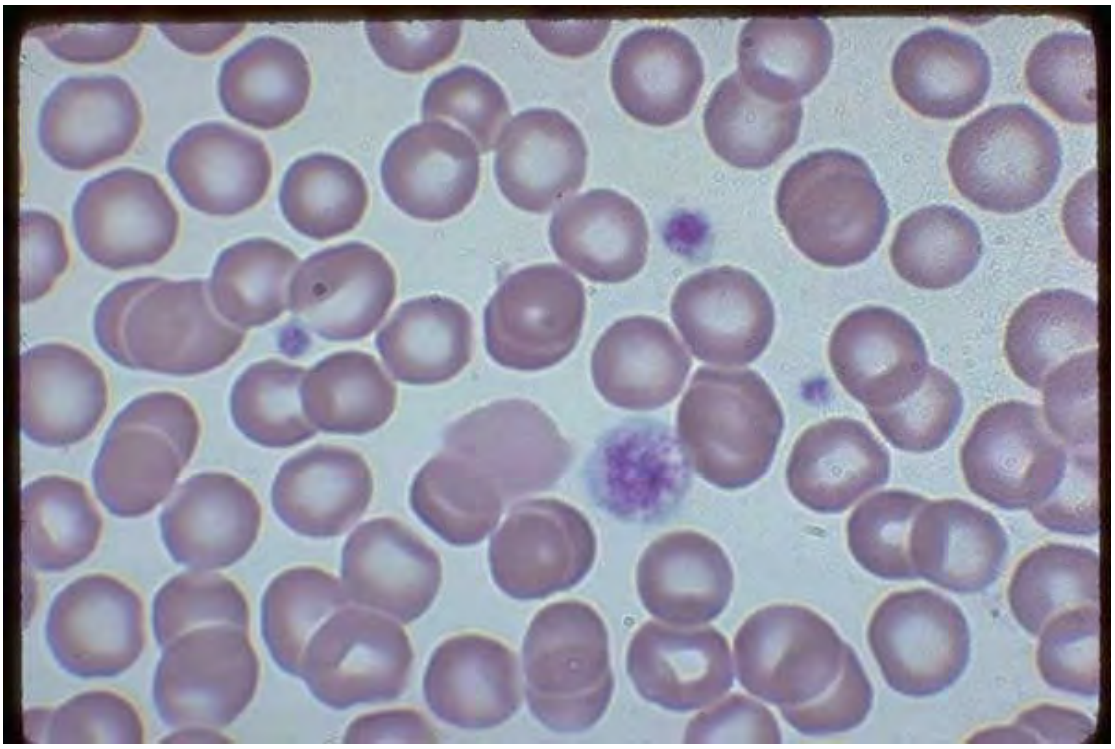
Normal Platelets , Normal EDTA anticoagulated blood 2.

Normal Platelets scattered throughout the field. The normal average number of platelets in a proper field ranges from 8 to 15. In EDTA anticoagulated samples they are more evenly distributed, are usually rounded up and may be slightly swollen and/or slightly degranulated. One small mature lymphocyte. Normal RBC. Normal EDTA anticoagulated blood - 100X



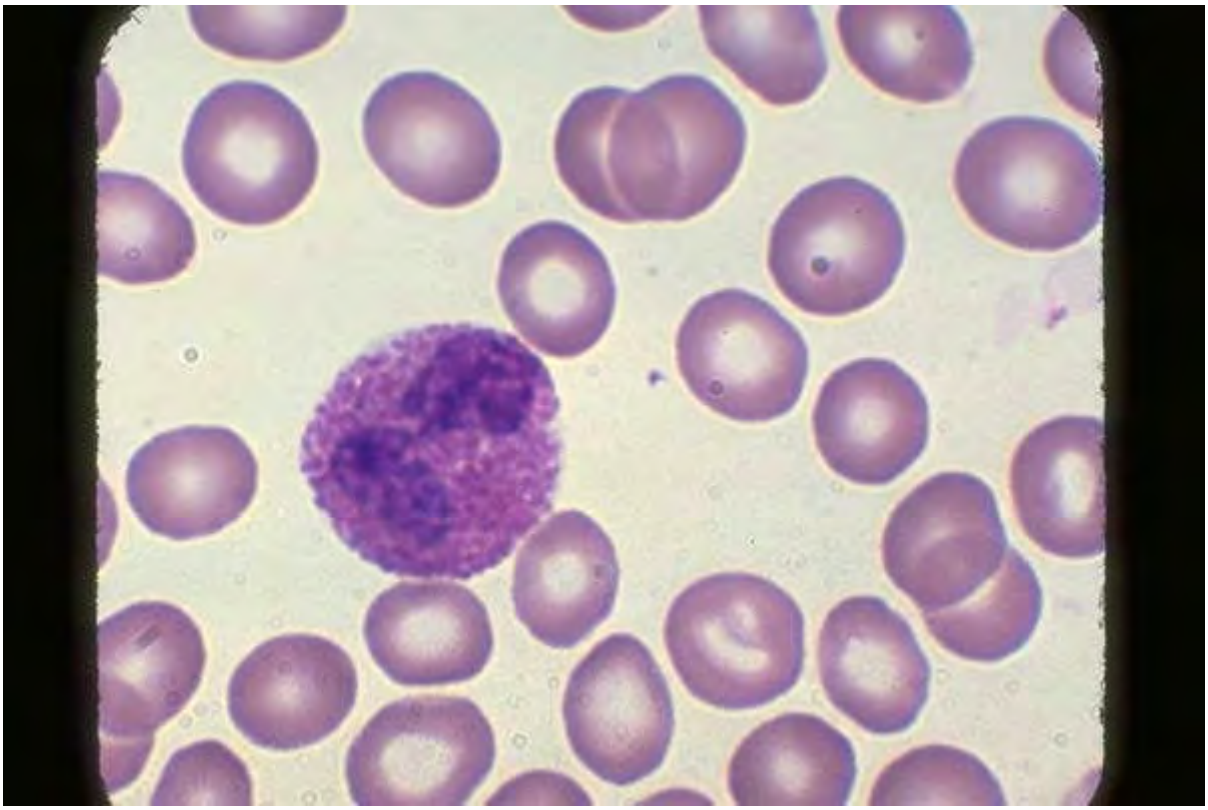
Platelets, Megathrombocyte, EDTA anticoagulated blood

Two small Platelets, 1 large platelet and 1 Megathrombocyte. Normal EDTA anticoagulated blood - 100X



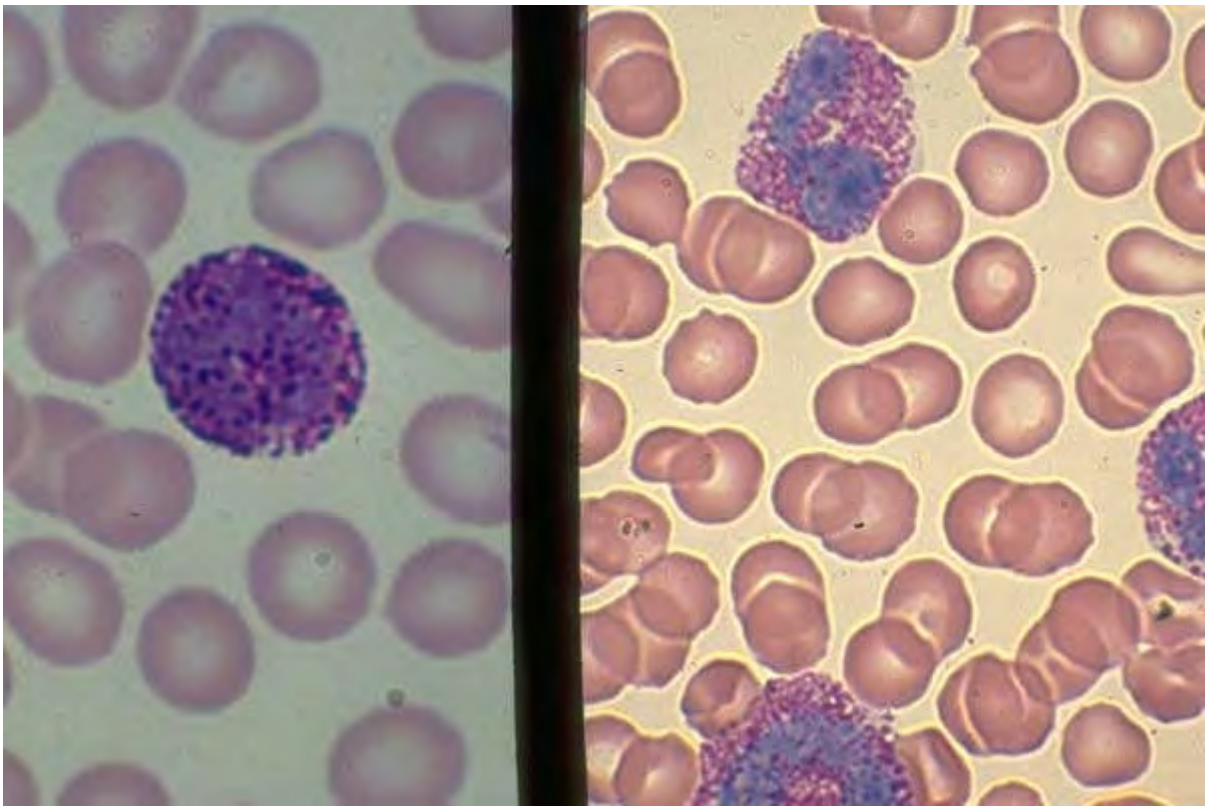
Eosinophil, Normal blood

One eosinophil - mature. Normal blood - 100X



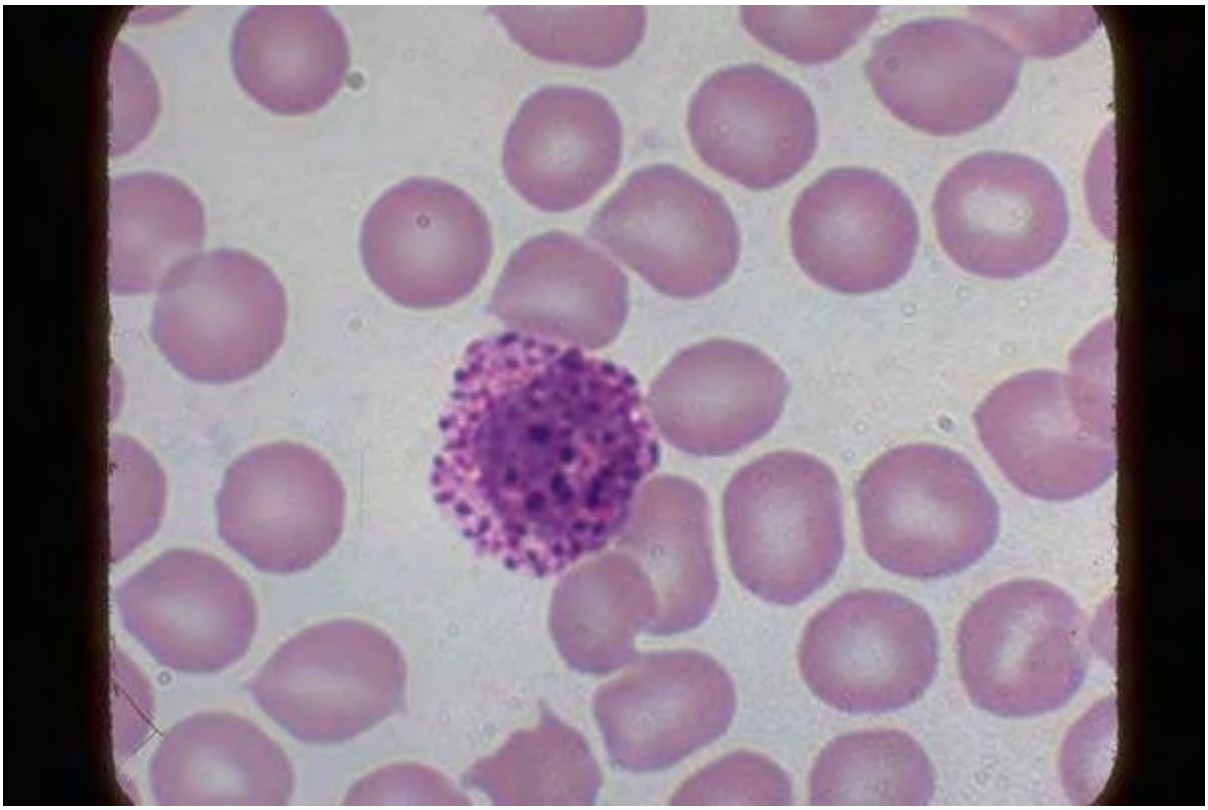
Basophil, eosinophils, Normal blood

One basophil, 3 eosinophils; composite. Normal blood - 100X



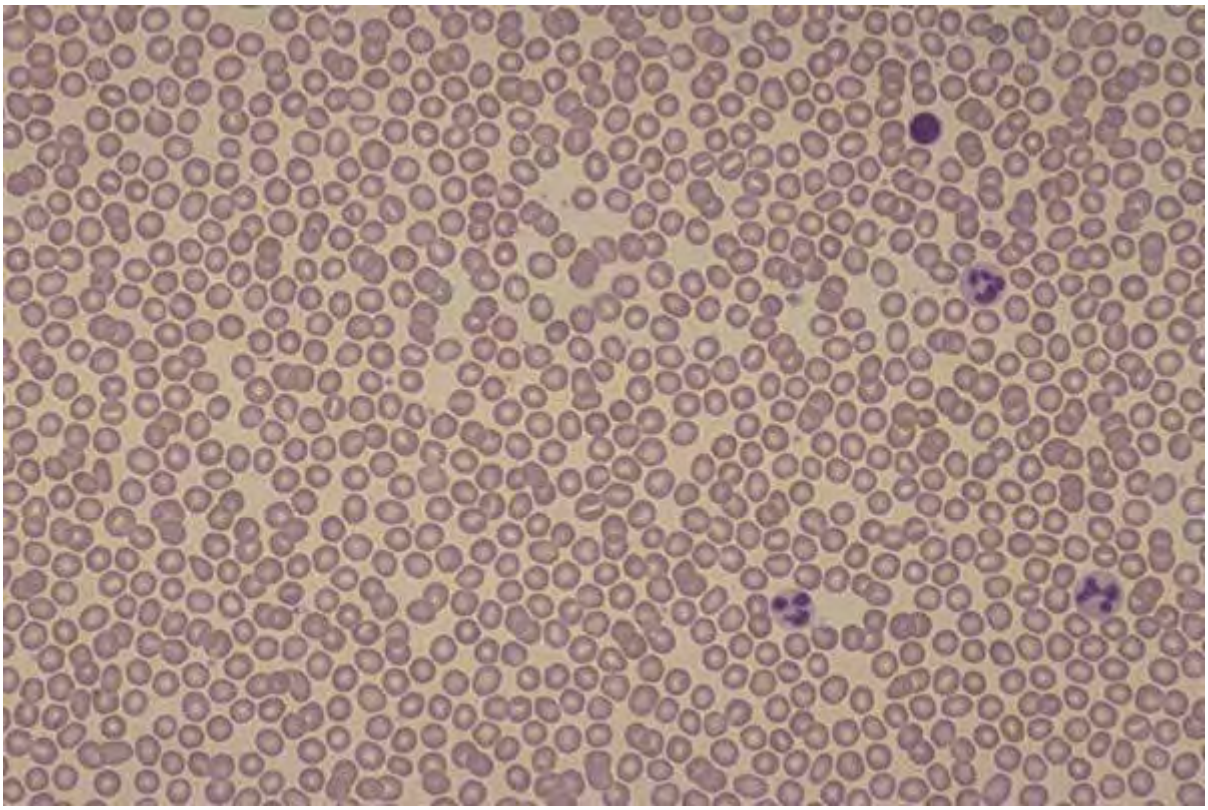
Basophil

One mature basophil. Normal blood - 100X



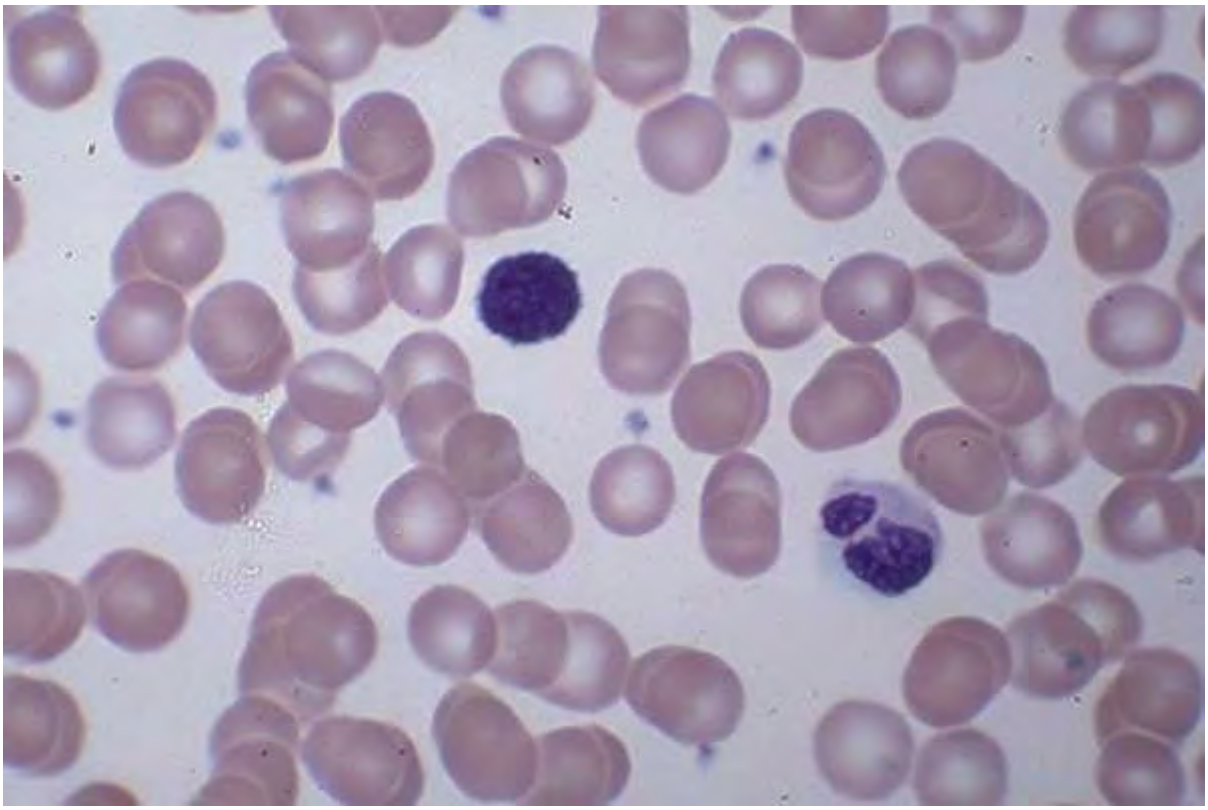
Normal blood

Normal blood - 20X



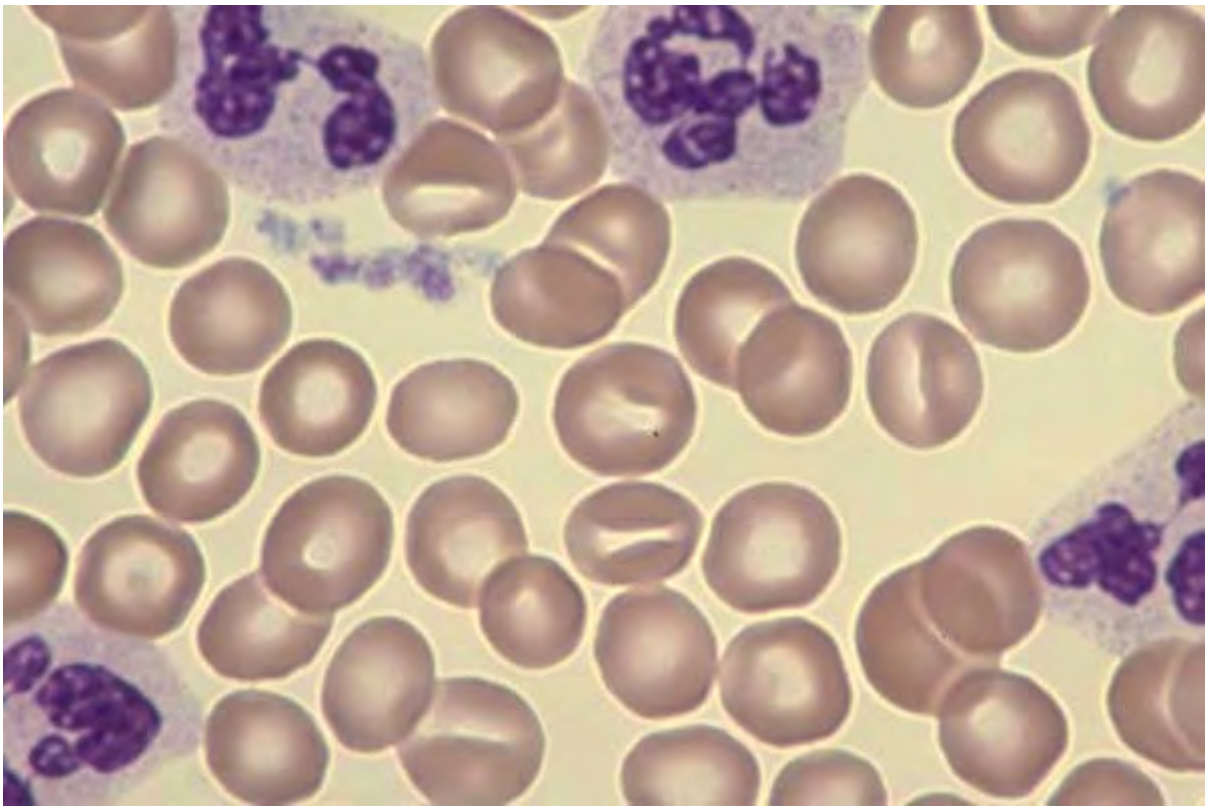
Normal blood - dense area, lyphocyte, segmented neutrophil

Normal blood - 1 lymphocyte and segmented neutrophil - 100X



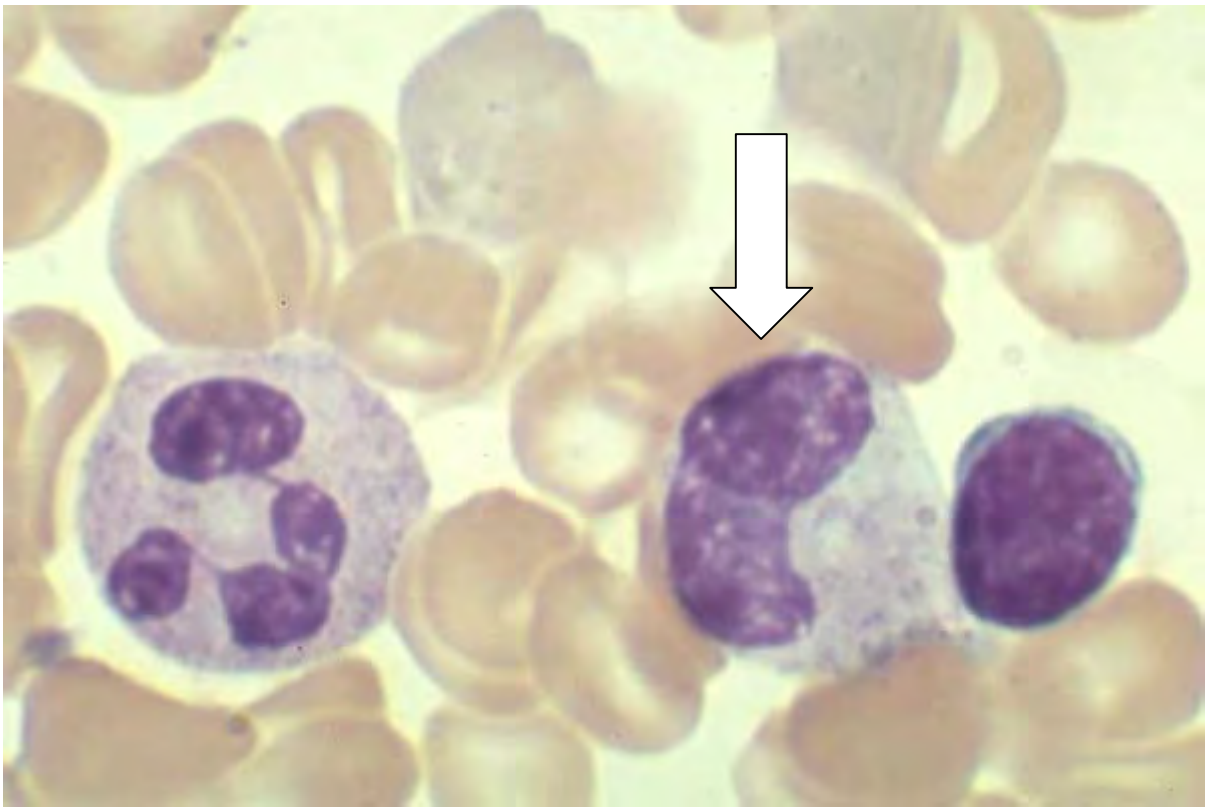
Normal blood, segmented neutrophils

Normal blood - 4 segmented neutrophils - 100X

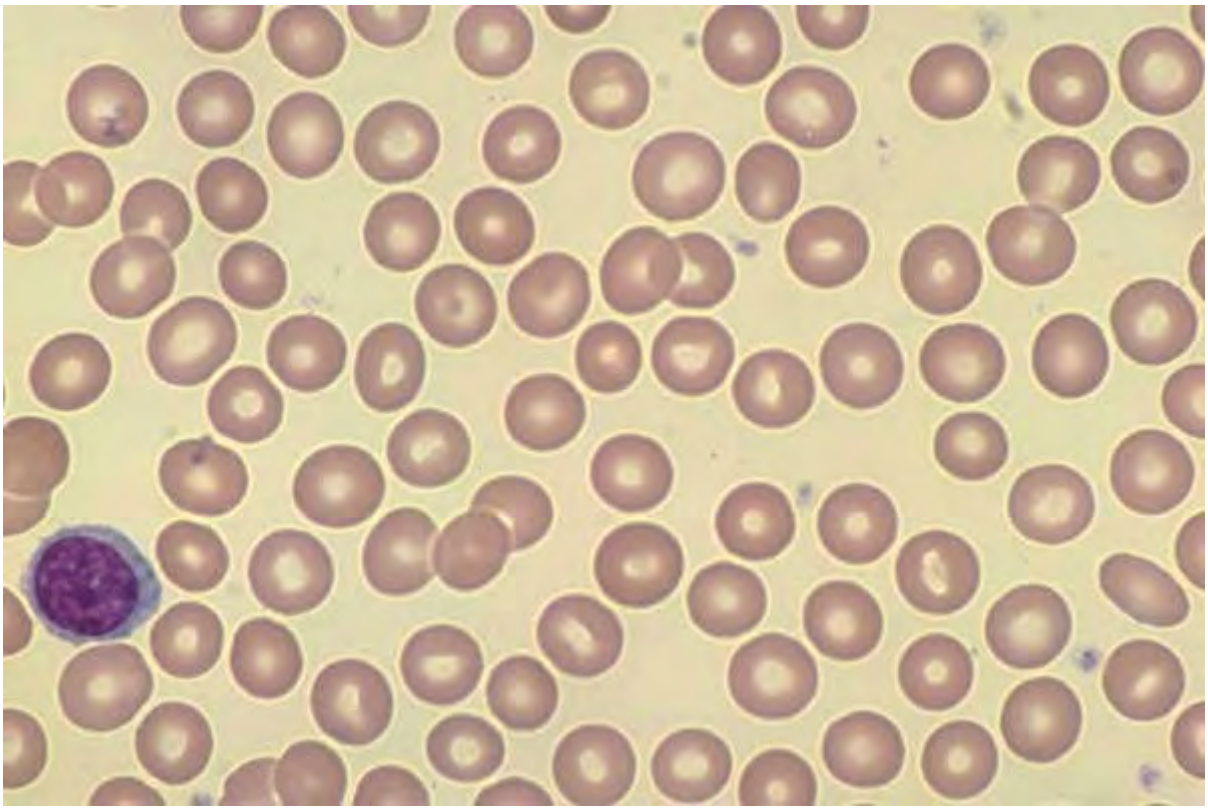


Segmented and band neutrophils, lymphocyte, normal blood

One segmented neutrophil, 1 band neutrophil (arrow), 1 lymphocyte. Normal blood - 100X

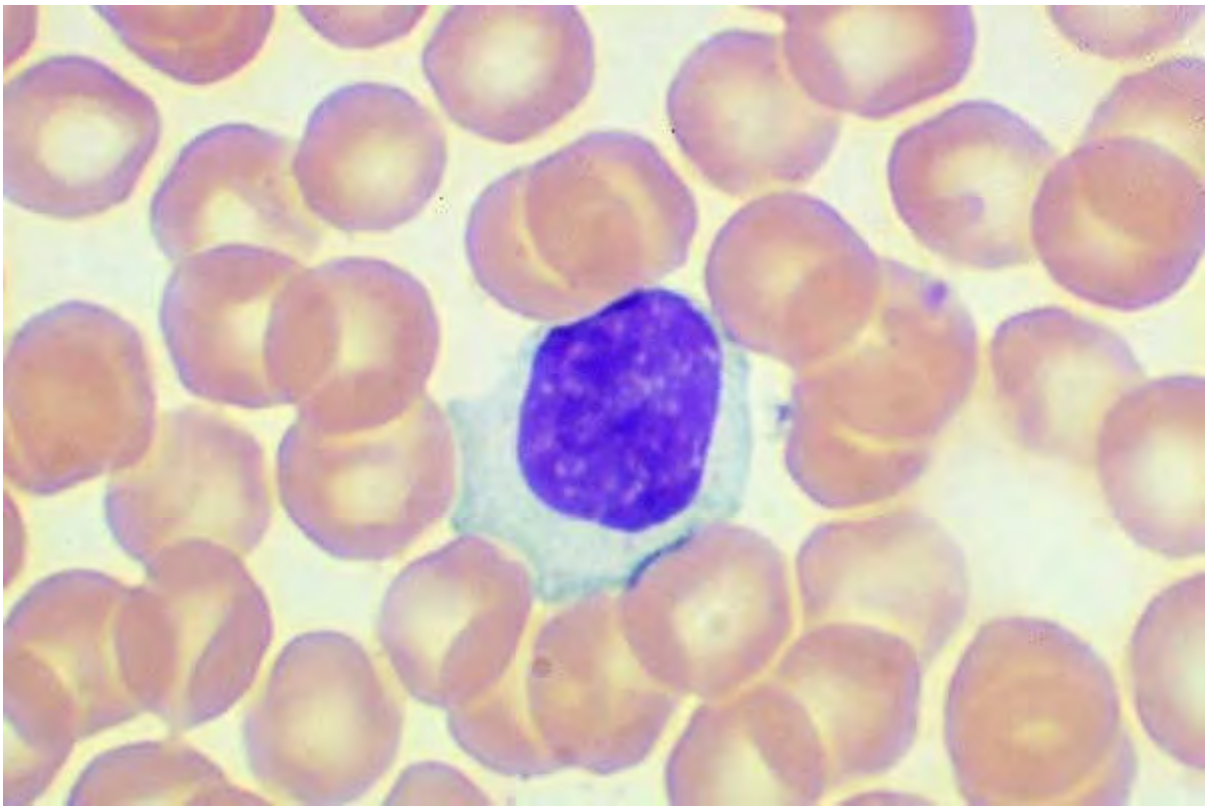


One lymphocyte; Normal blood - 100X



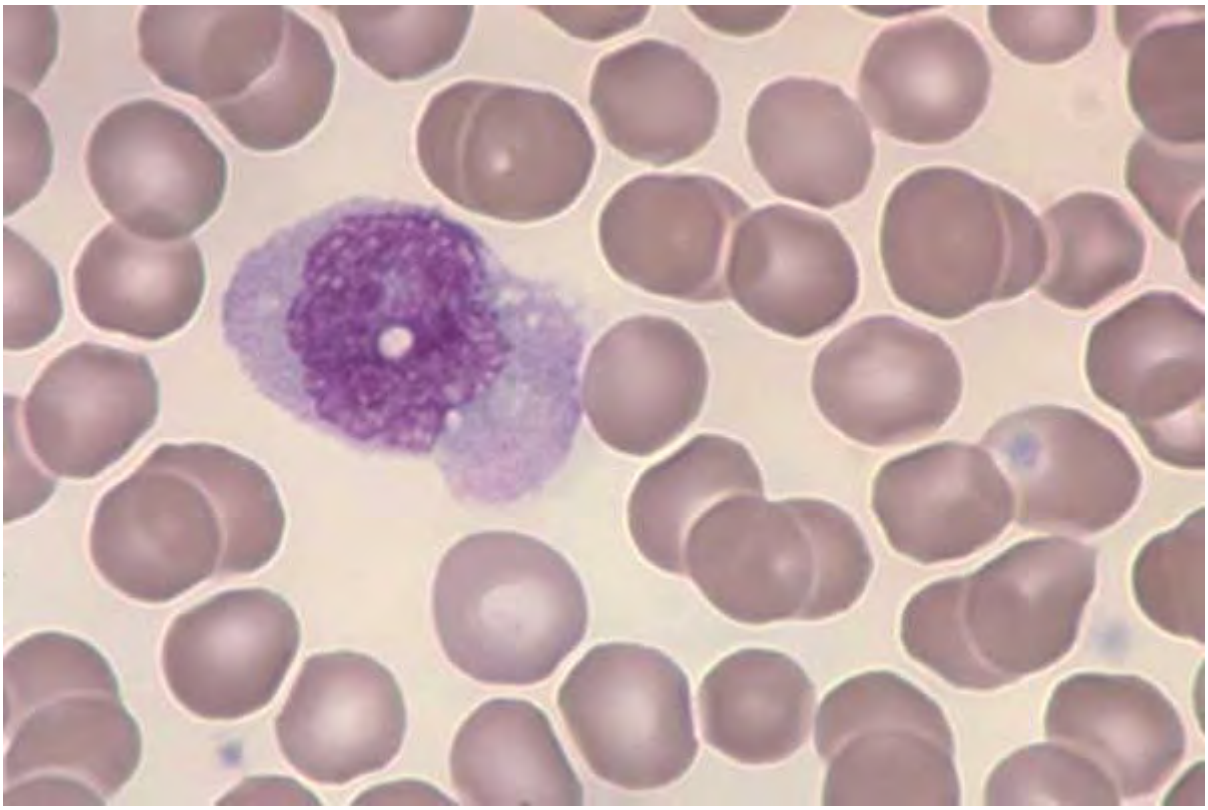
Very large, reactive lymphocyte, normal blood

One very large lymphocyte (reactive). Normal blood - 100X



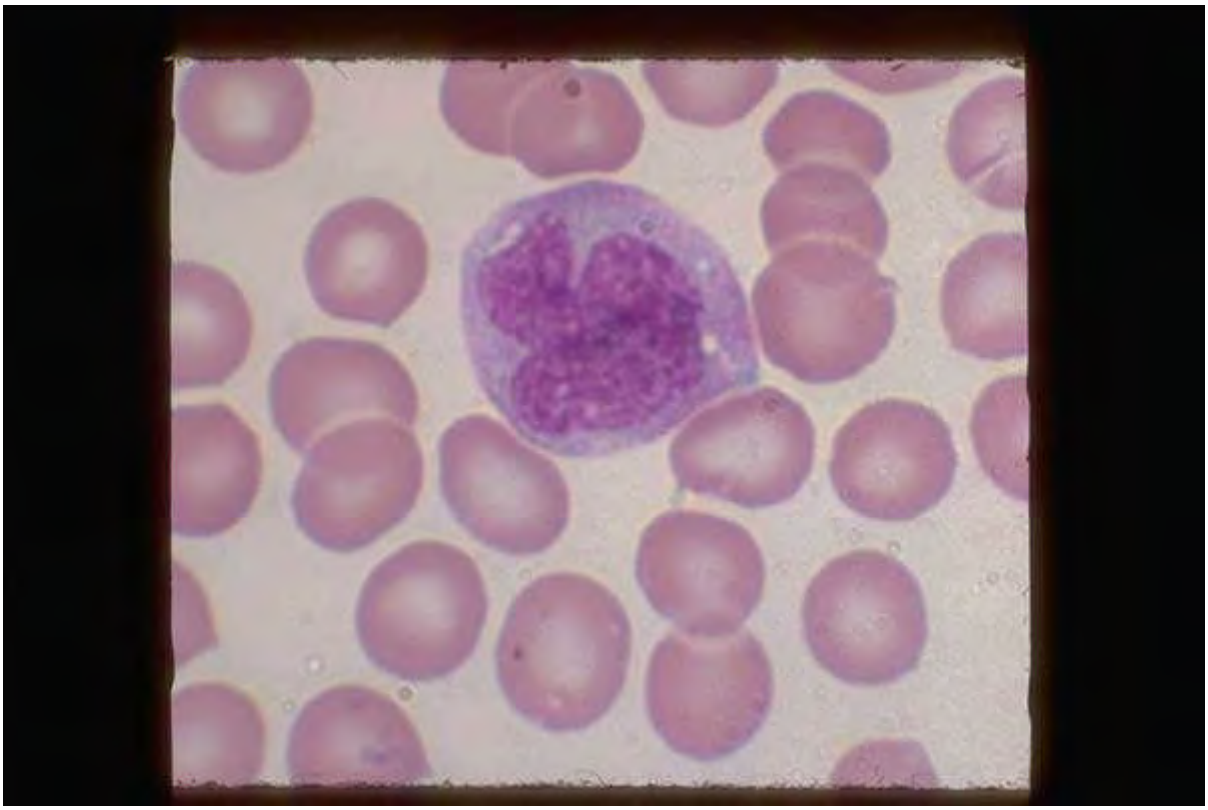
Monocyte, normal blood

One monocyte with vacuole in center of nucleus. Normal blood - 100X



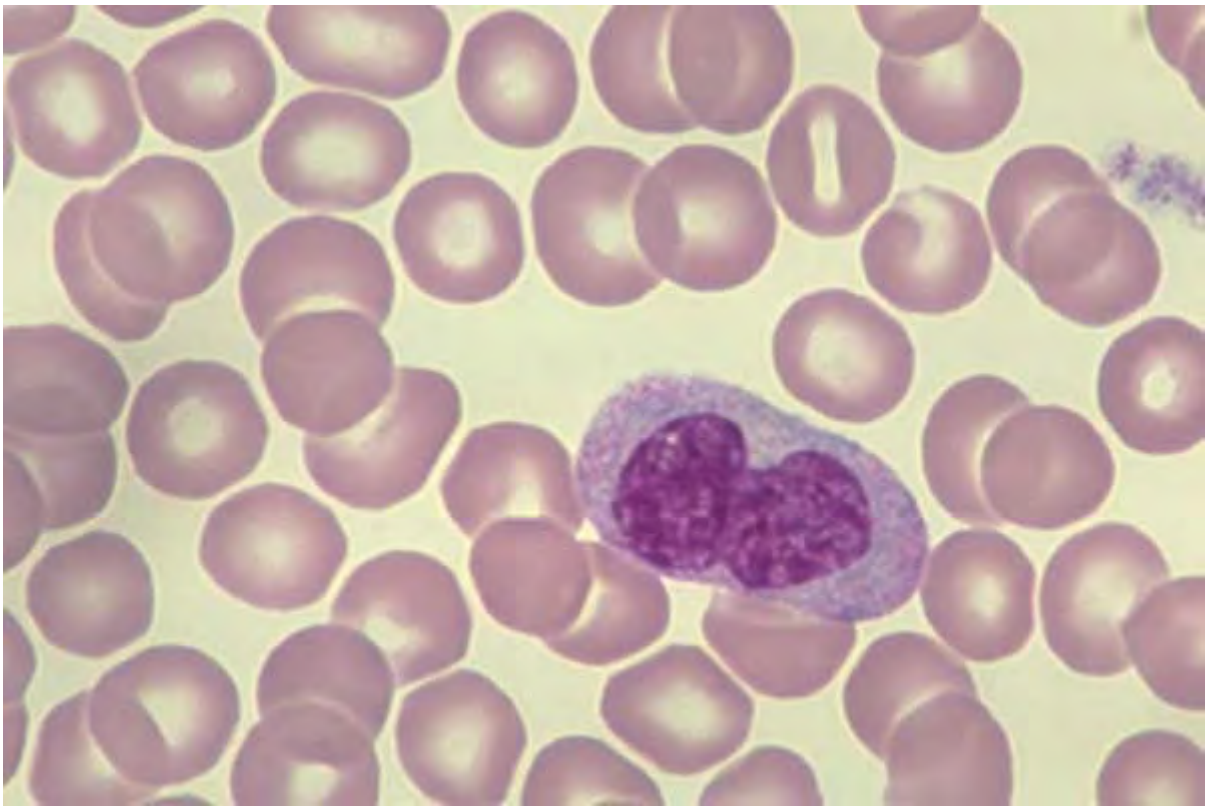
Monocyte with several small vacuoles, normal blood

One monocyte with several small vacuoles. Normal blood - 100X



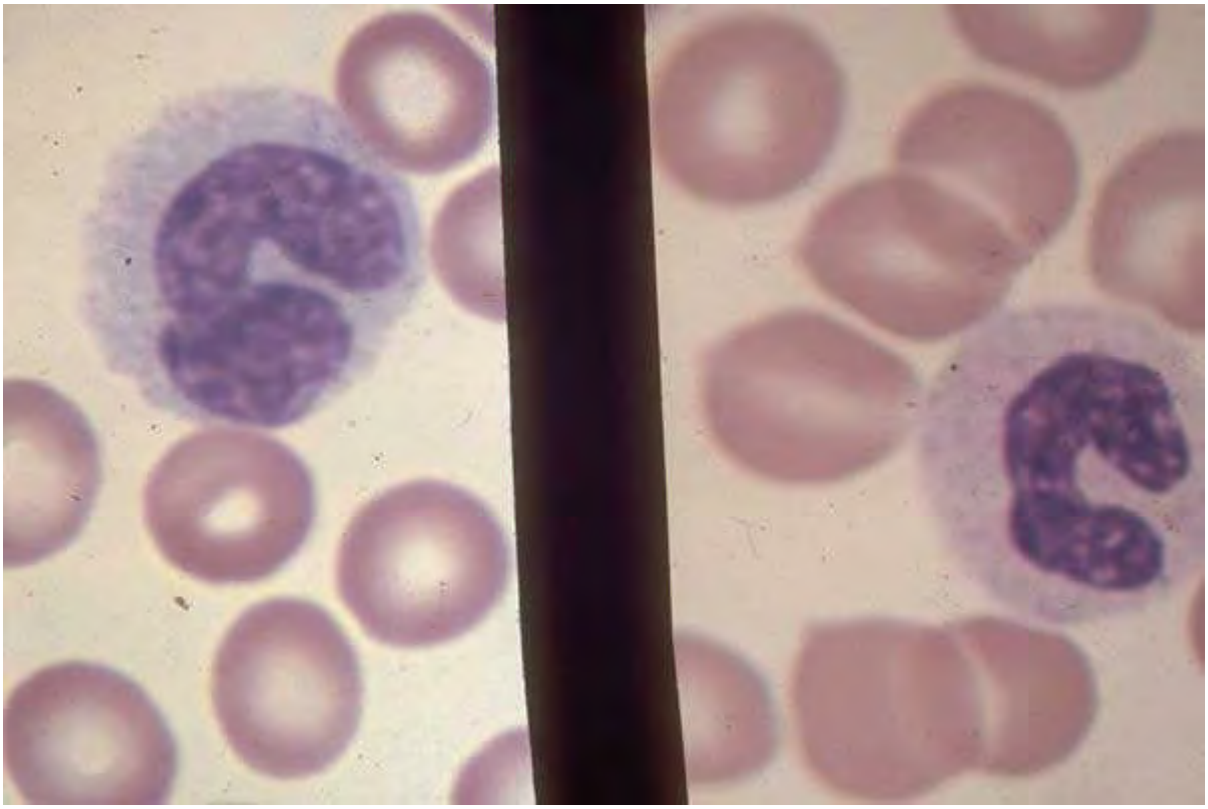
Monocyte, normal blood

One monocyte (sl. oval - elliptical shape). Normal blood - 100X



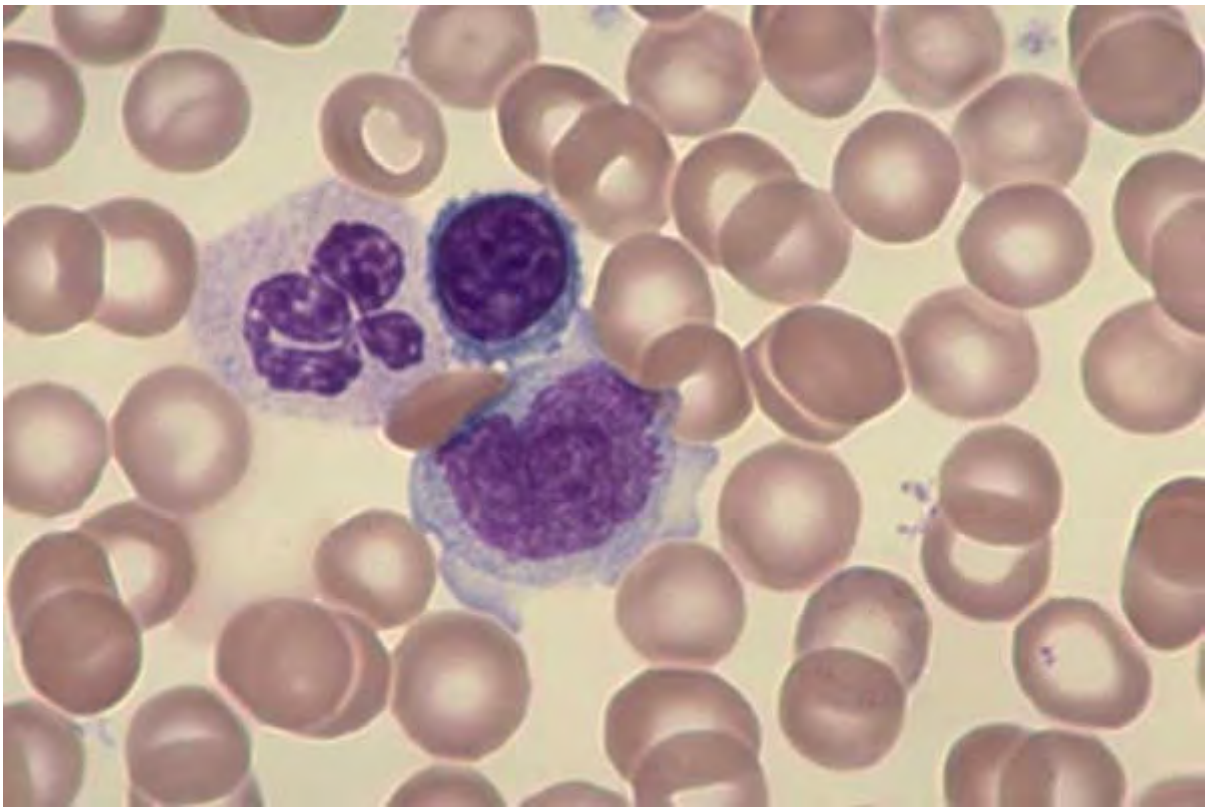
Monocyte, band neutrophil, normal blood

One monocyte with a band shaped nucleus in left frame, 1 band neutrophil in right frame. Compare nuclear chromatin and cytoplasmic color. Normal blood - 100X



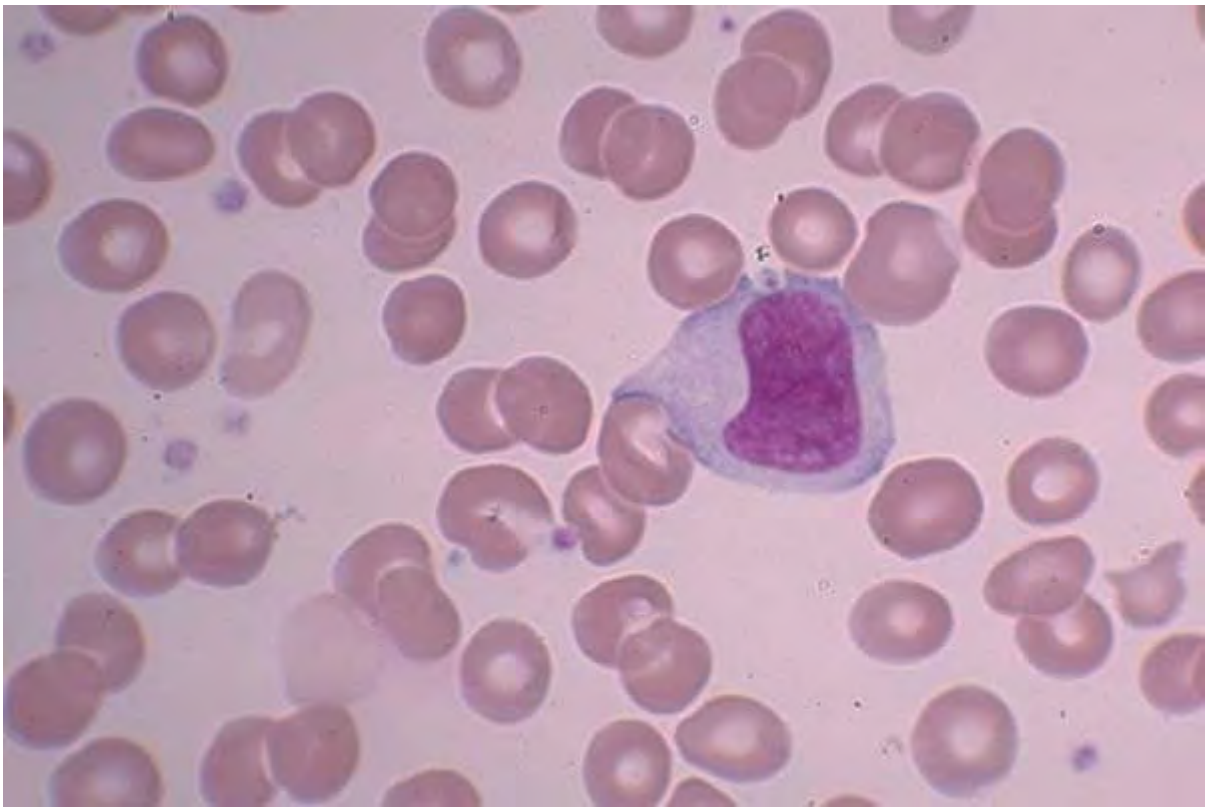
Segmented neutrophil, lymphocyte, monocyte, normal blood

One segmented neutrophil, 1 small lymphocyte with spiculated cytoplasm, 1 monocyte with extended cytoplasm; Normal blood - 100X



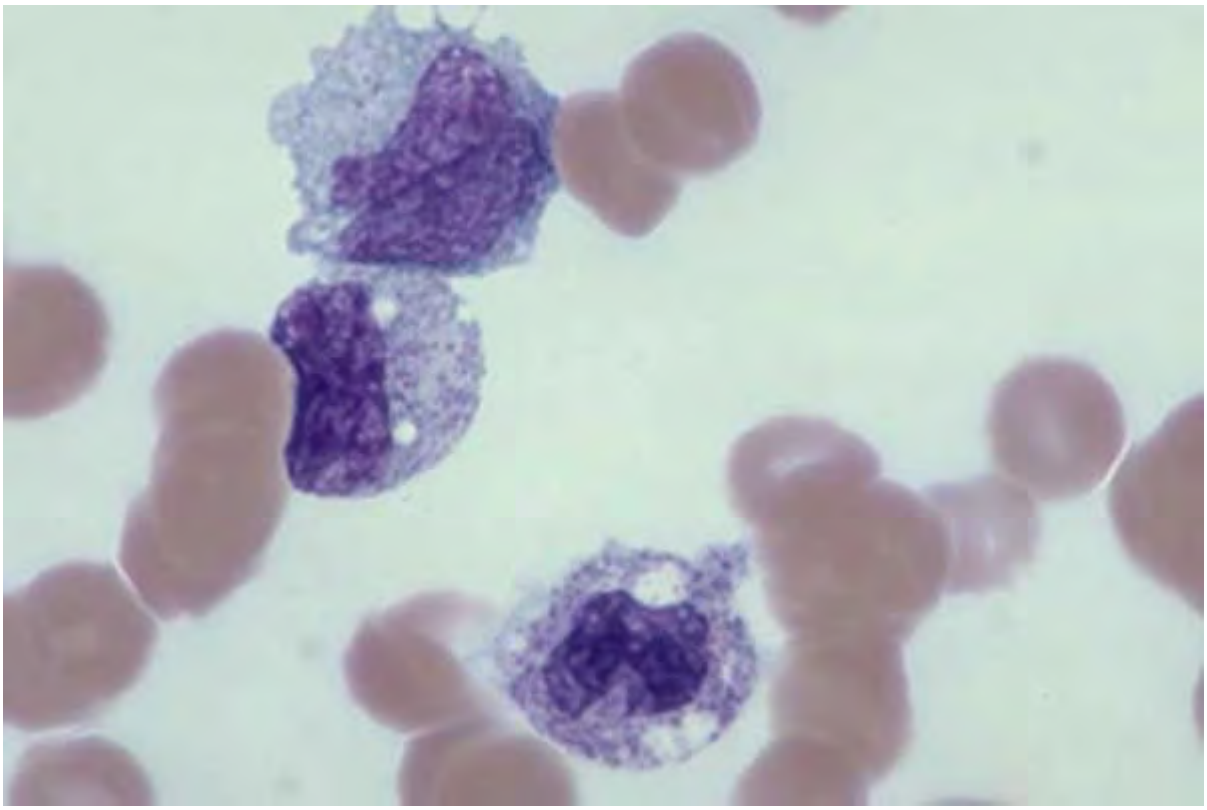
Young monocyte, normal blood

One young monocyte with immature chromatin and nucleoli. Normal blood - 100X



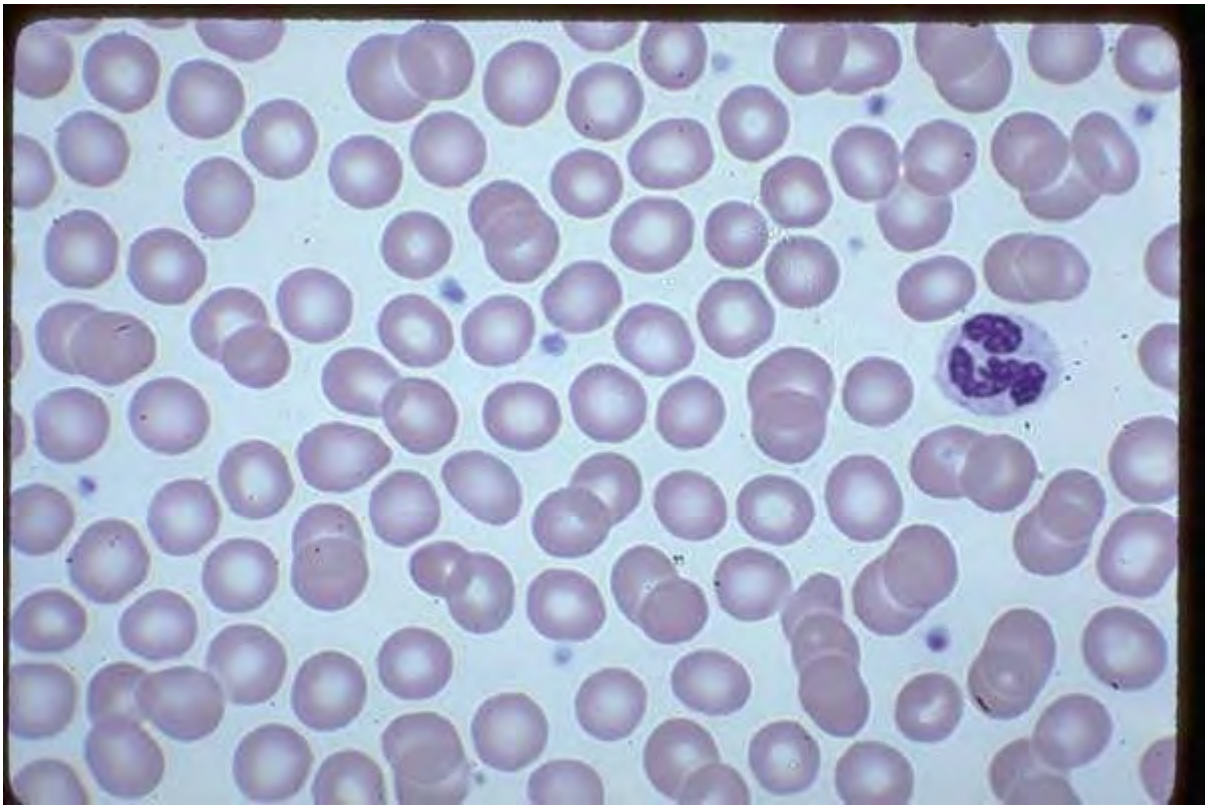
Active monocyte, toxic change neutrophils, sepsis

One active monocyte (top), 2 toxic change neutrophils. Sepsis - 100X



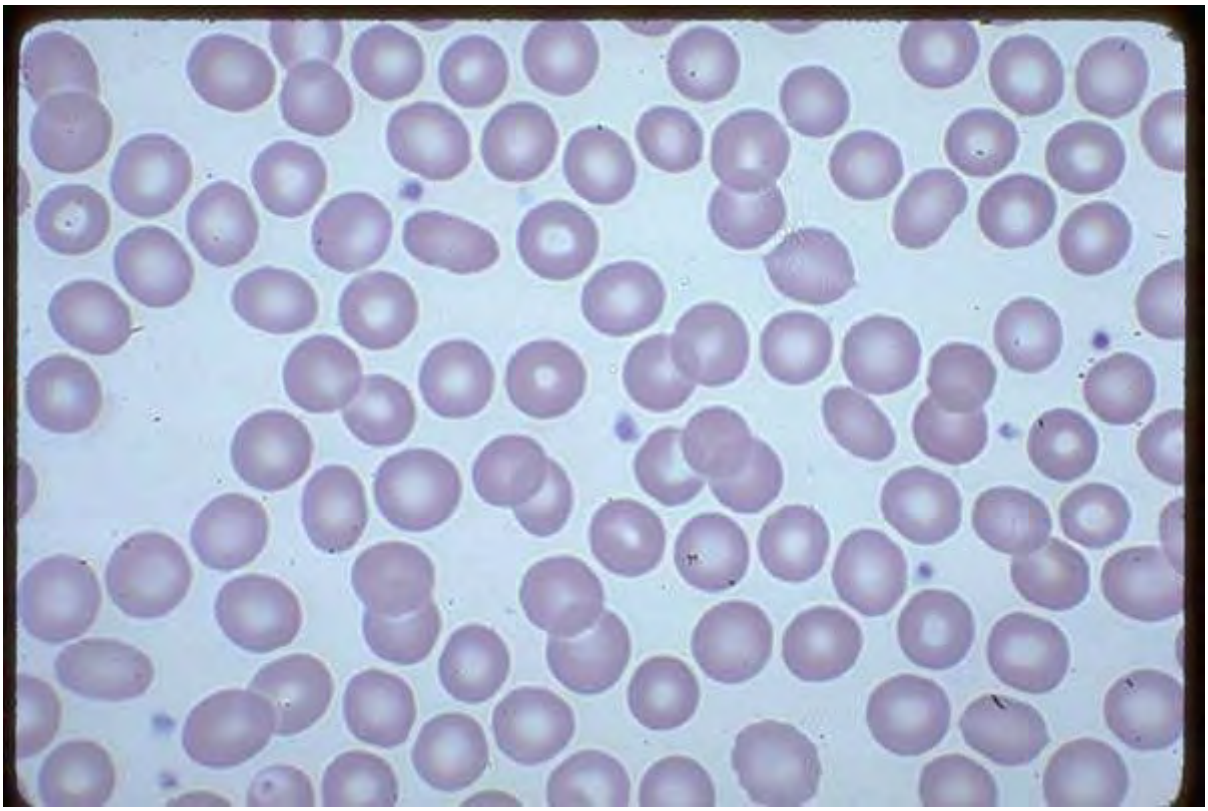
Normal Platelets , Proper Thickness, Field to Examine

Normal Platelets scattered throughout the field. There are slightly fewer than in the previous fields, but within normal numbers. This is a Proper Thickness of a Field to Examine, where the red cells are just touching each other or barely overlapping. Count the number of platelets in at least 10 similar fields and figure the average number per field to derive a platelet estimate. The Red Cells are Normocytic, Normochromic. The white cell is a Normal Mature Neutrophil. Normal blood - 100X.



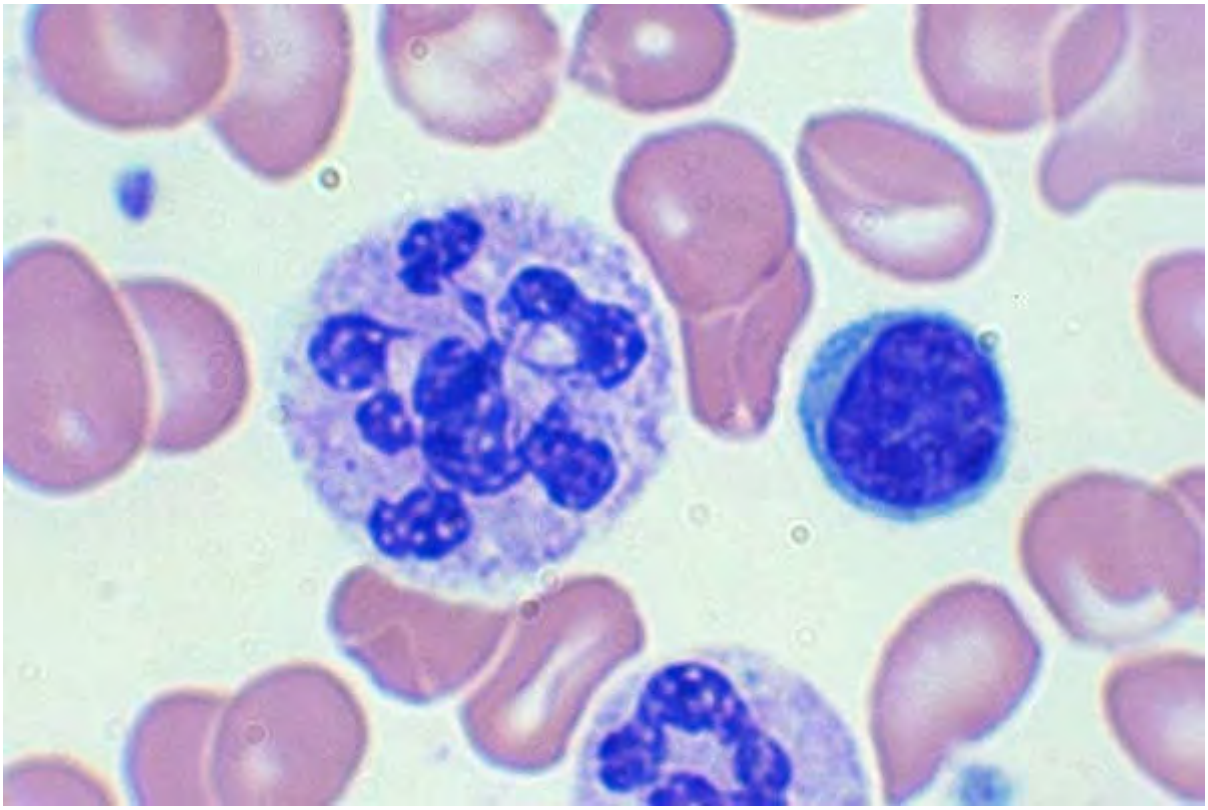
Normal Platelets, Normocytic, normochromic RBC, Normal EDTA

Normal Platelets in number and morphology. Normocytic, normochromic RBC. Normal EDTA anticoagulated blood - 100X



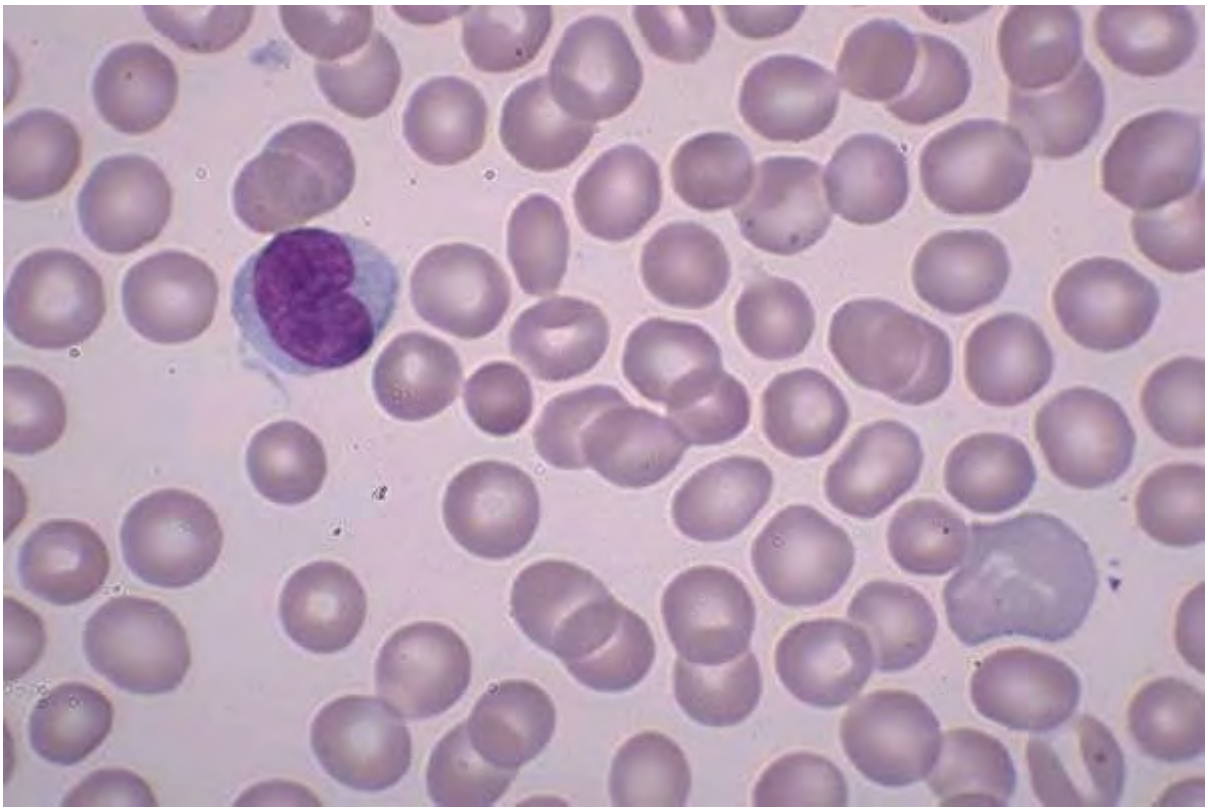
Hypersegmented neutrophil (possible artefact) , buffy coat of pernicious anemia

One hypersegmented neutrophil, 1 possible hypersegmented neutrophil, 1 small lymphocyte, macrocytic red cells. Buffy coat of pernicious anemia blood - 100X



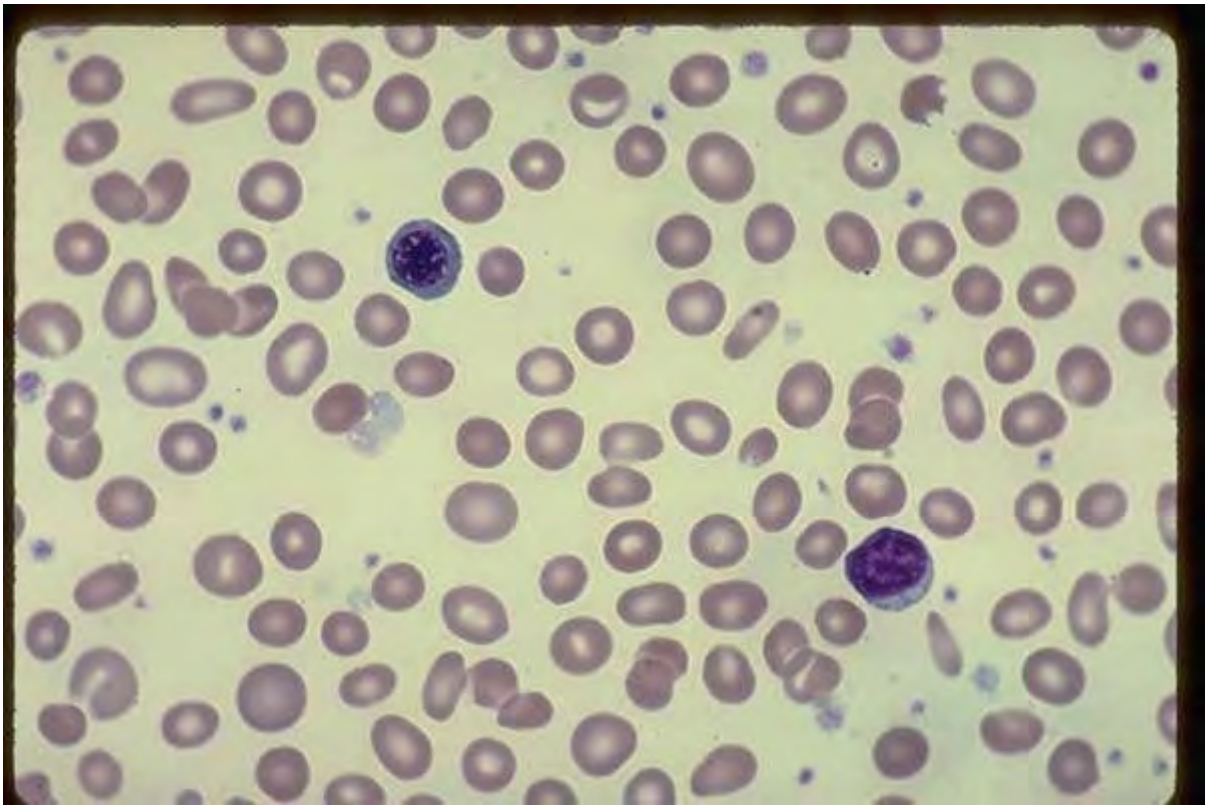
Lymphocyte, polychromatophilic RBC

One lymphocyte with kidney shaped nucleus, 1 polychromatophilic RBC in lower quadrant (reticulocyte) - 100X



nRBC, Mature Lymphocyte, AML

One NRBC at upper left and one Mature Lymphocyte at lower right. Many platelets are scattered throughout the field. An Abnormal Megathrombocyte, which is huge, irregular in shape and agranular, is at the left center. Acute Megakaryocytic Leukemia (M-7) untreated. Blood - 50X



ISIS Reference Values
Scientific name: PAN TROGLODYTES
Common Name: Chimpanzee

		Mean S.D.	Min. Max. (N)
WBC	*10^9/L	10.92 + 4.478	2.900 33.80(852)
RBC	*10^12/L	5.49 + 0.72	2.96 8.80 (717)
HGB	GM/L	142 + 17.0	76.0 199 (716)
HCT/PCV	L/L	0.438 + 0.055	0.246 0.840(869)
MCH	MMOL/L	26.0 + 2.4	13.4 35.9 (675)
MCHC	gm/L	328 + 16.0	204 403 (710)
MCV	fL	79.8 + 8.6	42.3 145.2 (708)
SEGS	*10^9/L	7.072 + 4.205	0.069 28.80(829)
BANDS	*10^9/L	0.262 + 0.450	0.000 3.550(156)
LYMPHOCYTES	*10^9/L	3.251 + 1.950	0.134 19.20(837)
MONOCYTES	*10^9/L	0.391 + 0.349	0.000 3.350(697)
EOSINOPHILS	*10^9/L	0.242 + 0.220	0.000 2.006(528)
BASOPHILS	*10^9/L	0.068 + 0.054	0.000 0.223(113)
NRBC	/100 WBC	0 + 1	0 3 (37)
PLATE. CNT.	*10^12/L	0.245 + 0.081	0.0 0.492 (228)
RETICS	%	0.1 + 0.1	0.0 0.3 (40)
GLUCOSE	MMOL/L	4.61 + 1.33	1.44 12.4 (758)
BUN	MMOL/L	3.93 + 1.43	0.0 12.1 (769)
CREAT.	UMOL/L	88.4 + 44.2	0.0 946 (724)
URIC ACID	MMOL/L	0.161 + 0.060	0.0 0.333 (326)
CA	MMOL/L	2.33 + 0.175	1.83 3.25 (754)
PHOS	MMOL/L	1.36 + 0.485	0.452 3.10 (706)
NA	MMOL/L	141 + 5	116 179 (677)
K	MMOL/L	3.9 + 0.6	0.0 7.6 (681)
CL	MMOL/L	103 + 4	90 117 (656)
IRON	UMOL/L	17.2 + 8.41	4.48 55.8 (60)
MG	MMOL/L	0.691 + 0.086	0.494 0.831 (19)
HCO3	MMOL/L	26.0 + 4.3	12.0 36.0 (79)
CHOL	MMOL/L	5.52 + 1.30	2.23 10.8 (738)
TRIG	MMOL/L	1.12 + 0.542	0.283 3.14 (326)
T.PROT. (C)	GM/L	73.0 + 7.00	54.0 95.0 (678)
ALBUMIN (C)	GM/L	37.0 + 4.00	25.0 50.0 (617)
GLOBULIN (C)	GM/L	36.0 + 7.00	19.0 62.0 (616)
AST (SGOT)	U/L	23 + 11	7 72 (675)
ALT (SGPT)	U/L	30 + 13	7 97 (715)
T. BILI.	UMOL/L	5.13 + 3.42	0.0 34.2 (722)
D. BILI	UMOL/L	1.71 + 1.71	0.0 5.13 (170)
I. BILI.	UMOL/L	3.42 + 1.71	0.0 10.3 (170)
AMYLASE	U/L	7.22 + 4.26	0.0 22.6 (350)
ALK.PHOS.	U/L	286 + 354	15 3528 (758)
LDH	U/L	409 + 238	77 1537 (446)
CPK	U/L	239 + 204	24 1343 (306)
OSMOLARITY	OSMOL/L	0.288 + 0.008	0.271 0.300 (18)
ALPHA-1 GLOB	GM/L	0.210 + 0.654	0.002 2.07 (10)
ALPHA-2 GLOB	GM/L	0.677 + 2.12	0.004 6.72 (10)

BETA GLOB. GM/L	1.04 + 3.08	0.007 9.25 (9)
Body Temperature:	36.7 + 1.0	34.0 39.4 (482)
CO2 MMOL/L	24.7 + 4.9	12.0 46.5 (153)
ESR	5 + 7	0 24 (15)
FIBRINOGEN G/L	3.33 + 0.580	3.00 4.00 (3)
FREE T3 NMOL/L	1617 + 0.0	1617 1617 (1)
GGT U/L	29 + 15	7 120 (288)
HDL MMOL/L	1.50 + 0.596	0.622 3.00 (24)
LIPASE U/L	7.51 + 5.00	0.0 26.7 (99)
LDL MMOL/L	2.85 + 0.932	1.71 5.10 (21)
PROGESTERONE NMOL/L	6.82 + 21.3	0.006 67.6 (10)
TESTOSTERONE NMOL/L	8099 + 0.0	8099 8099 (1)
TOT. T4(RIA) NMOL/L	112 + 40.0	46.4 181 (18)
TOT. T3(RIA) NMOL/L	2.76 + 0.571	2.22 3.36 (3)
ALBUMIN (E) GM/L	42.0 + 0.0	42.0 42.0 (1)
GAMMA GLOB GM/L	12.0 + 0.0	12.0 12.0 (1)

Scientific name: GORILLA GORILLA
Common Name: Gorilla

	Mean S.D.	Min. Max. (N)
WBC *10^9/L	8.236 + 3.566	2.500 26.90(902)
RBC *10^12/L	4.62 + 0.58	3.11 6.66 (807)
HGB GM/L	124 + 14.0	77.0 190 (825)
HCT/PCV L/L	0.389 + 0.044	0.270 0.562(937)
MCH MMOL/L	27.0 + 2.3	14.6 38.4 (781)
MCHC gm/L	322 + 17.0	211 452 (812)
MCV fL	83.5 + 7.4	50.0 125.0 (796)
SEGS *10^9/L	5.311 + 3.182	0.042 21.10(840)
BANDS *10^9/L	0.178 + 0.225	0.000 1.240(246)
LYMPHOCYTES *10^9/L	2.318 + 1.291	0.025 10.10(846)
MONOCYTES *10^9/L	0.383 + 0.261	0.000 1.576(764)
EOSINOPHILS *10^9/L	0.194 + 0.201	0.000 2.020(561)
BASOPHILS *10^9/L	0.045 + 0.054	0.000 0.306(147)
NRBC /100 WBC	0 + 0	0 1 (85)
PLATE. CNT. *10^12/L	0.188 + 0.109	0.001 0.657(381)
RETICS %	0.2 + 0.3	0.0 1.7 (62)
GLUCOSE MMOL/L	4.22 + 0.888	1.67 8.38 (905)
BUN MMOL/L	3.93 + 1.43	0.0 10.7 (901)
CREAT. UMOL/L	97.2 + 35.4	35.4 265 (860)
URIC ACID MMOL/L	0.077 + 0.036	0.0 0.196 (528)
CA MMOL/L	2.38 + 0.125	1.95 2.83 (877)
PHOS MMOL/L	1.36 + 0.291	0.614 2.49 (830)
NA MMOL/L	137 + 3	127 148 (851)
K MMOL/L	4.2 + 0.5	2.6 6.5 (856)
CL MMOL/L	101 + 4	76 115 (840)
IRON UMOL/L	17.7 + 6.98	6.27 46.7 (160)
MG MMOL/L	0.666 + 0.128	0.370 1.14 (131)
HCO3 MMOL/L	24.1 + 3.2	17.0 33.0 (78)
CHOL MMOL/L	7.36 + 2.15	2.64 18.1 (863)

TRIG MMOL/L	1.34 + 0.791	0.226 7.01 (574)
T.PROT. (C) GM/L	73.0 + 7.00	54.0 95.0 (798)
T.PROT. (R) GM/L	86.0 + 8.00	68.0 91.0 (8)
ALBUMIN (C) GM/L	37.0 + 4.00	26.0 48.0 (744)
GLOBULIN (C) GM/L	36.0 + 7.00	18.0 66.0 (744)
AST (SGOT) U/L	33 + 18	7 133 (861)
ALT (SGPT) U/L	29 + 17	4 118 (833)
T. BILI. UMOL/L	8.55 + 5.13	0.0 39.3 (877)
D. BILI UMOL/L	1.71 + 1.71	0.0 8.55 (280)
I. BILI. UMOL/L	6.84 + 3.42	0.0 22.2 (279)
AMYLASE U/L	5.18 + 3.15	0.0 19.4 (383)
ALK.PHOS. U/L	437 + 356	47 2550 (879)
LDH U/L	603 + 445	121 2812 (634)
CPK U/L	363 + 407	35 3930 (356)
OSMOLARITY OSMOL/L	0.278 + 0.014	0.259 0.338 (32)
ALPHA-1 GLOB GM/L	0.003 + 0.002	0.001 0.005 (5)
ALPHA-2 GLOB GM/L	0.008 + 0.003	0.005 0.012 (5)
BETA GLOB. GM/L	0.011 + 0.006	0.007 0.020 (5)
Body Temperature:	37.0 + 0.8	35.0 39.0 (582)
CO2 MMOL/L	24.6 + 5.8	12.0 49.0 (389)
CORTISOL NMOL/L	439 + 127	292 516 (3)
ESR	3 + 3	0 18 (33)
FIBRINOGEN G/L	3.36 + 1.96	1.00 8.00 (16)
FREE T3 NMOL/L	728 + 1143	60.1 2048 (3)
GGT U/L	35 + 54	0 490 (445)
HDL MMOL/L	2.41 + 0.907	0.751 5.18 (41)
LIPASE U/L	3.34 + 5.56	0.0 33.1 (149)
LDL MMOL/L	3.94 + 1.27	1.42 6.63 (43)
PROGESTERONE NMOL/L	0.204 + 0.366	0.010 1.19 (11)
TESTOSTERONE NMOL/L	687 + 793	0.069 1385 (4)
TOT. T4(RIA) NMOL/L	76.1 + 36.1	32.3 208 (55)
TOT. T3(RIA) NMOL/L	1.00 + 0.955	0.008 1.91 (3)
T3 UPTAKE %	40 + 4	29 47 (40)
GAMMA GLOB GM/L	6.00 + 0.0	6.00 6.00 (1)

Scientific name: MANDRILLUS LEUCOPHAEUS
Common Name: Drill

	Mean S.D.	Min. Max. (N)
WBC *10^9/L	7.834 + 3.997	3.250 24.60 (62)
RBC *10^12/L	4.62 + 0.50	3.58 6.23 (63)
HGB GM/L	119 + 12.0	95.0 149 (66)
HCT/PCV L/L	0.375 + 0.040	0.310 0.494 (66)
MCH MMOL/L	25.7 + 2.6	19.7 36.8 (63)
MCHC gm/L	317 + 16.0	269 355 (66)
MCV fL	81.3 + 7.8	66.3 113.5 (63)
SEGS *10^9/L	4.290 + 3.570	0.300 19.50 (57)
BANDS *10^9/L	0.630 + 1.269	0.000 3.480 (7)
LYMPHOCYTES *10^9/L	3.270 + 2.274	0.770 14.10 (56)
MONOCYTES *10^9/L	0.296 + 0.257	0.037 1.724 (54)
EOSINOPHILS *10^9/L	0.167 + 0.142	0.000 0.752 (38)
BASOPHILS *10^9/L	0.061 + 0.039	0.000 0.126 (17)
NRBC /100 WBC	0 + 0	0 1 (6)
PLATE. CNT. *10^12/L	0.219 + 0.149	0.002 0.563 (28)
GLUCOSE MMOL/L	4.50 + 1.17	2.05 7.88 (61)
BUN MMOL/L	5.71 + 1.43	2.50 8.93 (62)
CREAT. UMOL/L	97.2 + 26.5	53.0 194 (62)
URIC ACID MMOL/L	0.006 + 0.012	0.0 0.036 (31)
CA MMOL/L	2.30 + 0.200	1.75 2.73 (61)
PHOS MMOL/L	1.68 + 0.485	0.678 3.13 (60)
NA MMOL/L	148 + 4	137 155 (60)
K MMOL/L	3.8 + 0.4	2.9 5.2 (60)
CL MMOL/L	126 + 135	89 1111 (55)
MG MMOL/L	0.736 + 0.206	0.494 1.32 (37)
HCO3 MMOL/L	31.0 + 0.0	31.0 31.0 (1)
CHOL MMOL/L	3.57 + 0.855	1.74 5.96 (60)
TRIG MMOL/L	0.610 + 0.249	0.192 1.16 (58)
T.PROT. (C) GM/L	68.0 + 8.00	50.0 85.0 (53)
ALBUMIN (C) GM/L	43.0 + 6.00	28.0 56.0 (60)
GLOBULIN (C) GM/L	24.0 + 6.00	13.0 39.0 (60)
AST (SGOT) U/L	35 + 13	11 74 (62)
ALT (SGPT) U/L	46 + 23	7 138 (61)
T. BILI. UMOL/L	3.42 + 1.71	1.71 6.84 (60)
D. BILI UMOL/L	0.0 + 0.0	0.0 1.71 (39)
I. BILI. UMOL/L	3.42 + 1.71	0.0 6.84 (39)
AMYLASE U/L	47.4 + 16.1	23.1 97.7 (48)
ALK.PHOS. U/L	463 + 400	0 1512 (61)
LDH U/L	349 + 234	55 1330 (32)
CPK U/L	507 + 414	82 1870 (17)
Body Temperature:	38.1 + 0.9	36.0 40.0 (51)
CO2 MMOL/L	24.2 + 5.8	10.0 44.9 (46)
GGT U/L	85 + 36	0 173 (47)
LIPASE U/L	12.0 + 7.23	0.0 27.0 (47)
TOT. T4(RIA) NMOL/L	69.7 + 14.2	59.3 78.7 (2)

Scientific name: MANDRILLUS SPHINX
Common Name: Mandrill

		Mean S.D.	Min. Max. (N)
WBC	*10^9/L	10.24 + 4.431	3.100 31.10 (502)
RBC	*10^12/L	4.84 + 0.65	2.44 7.51 (415)
HGB	GM/L	117 + 11.0	83.0 161 (412)
HCT/PCV	L/L	0.377 + 0.040	0.265 0.540 (503)
MCH	MMOL/L	24.4 + 1.9	13.5 38.9 (396)
MCHC	gm/L	317 + 19.0	163 385 (408)
MCV	fL	76.9 + 6.5	50.7 124.2 (405)
SEGS	*10^9/L	5.845 + 4.043	0.340 26.40 (460)
BANDS	*10^9/L	0.508 + 0.696	0.000 4.500 (101)
LYMPHOCYTES	*10^9/L	3.755 + 2.220	0.272 14.80 (461)
MONOCYTES	*10^9/L	0.455 + 0.371	0.000 2.964 (403)
EOSINOPHILS	*10^9/L	0.188 + 0.154	0.000 1.020 (283)
BASOPHILS	*10^9/L	0.084 + 0.083	0.000 0.536 (111)
NRBC	/100 WBC	11 + 24	0 75 (21)
PLATE. CNT.	*10^12/L	0.290 + 0.077	0.135 0.682 (182)
RETICS	%	2.7 + 3.3	0.0 8.0 (6)
GLUCOSE	MMOL/L	5.22 + 1.89	0.0 12.0 (464)
BUN	MMOL/L	5.36 + 2.14	1.07 20.7 (462)
CREAT.	UMOL/L	106 + 35.4	0.0 221 (437)
URIC ACID	MMOL/L	0.018 + 0.048	0.0 0.607 (174)
CA	MMOL/L	2.35 + 0.225	1.70 3.23 (451)
PHOS	MMOL/L	1.55 + 0.646	0.420 3.97 (409)
NA	MMOL/L	148 + 5	120 165 (397)
K	MMOL/L	3.8 + 0.7	2.6 7.7 (392)
CL	MMOL/L	108 + 4	93 120 (397)
IRON	UMOL/L	22.6 + 6.27	14.0 47.3 (56)
MG	MMOL/L	0.646 + 0.251	0.234 1.07 (23)
HCO3	MMOL/L	21.9 + 4.7	11.0 29.0 (51)
CHOL	MMOL/L	3.81 + 1.06	0.0 7.87 (448)
TRIG	MMOL/L	0.893 + 0.396	0.226 2.98 (264)
T.PROT. (C)	GM/L	70.0 + 6.00	54.0 87.0 (414)
T.PROT. (R)	GM/L	72.0 + 8.00	59.0 84.0 (10)
ALBUMIN (C)	GM/L	45.0 + 6.00	27.0 70.0 (359)
GLOBULIN (C)	GM/L	25.0 + 6.00	11.0 44.0 (359)
AST (SGOT)	U/L	38 + 20	0 161 (443)
ALT (SGPT)	U/L	52 + 41	6 282 (415)
T. BILI.	UMOL/L	5.13 + 3.42	0.0 17.1 (434)
D. BILI	UMOL/L	1.71 + 1.71	0.0 5.13 (147)
I. BILI.	UMOL/L	3.42 + 1.71	0.0 12.0 (146)
AMYLASE	U/L	41.3 + 18.1	14.6 121 (159)
ALK.PHOS.	U/L	373 + 363	42 3039 (435)
LDH	U/L	648 + 648	64 3908 (272)
CPK	U/L	636 + 711	42 6760 (200)
OSMOLARITY	OSMOL/L	0.299 + 0.011	0.279 0.322 (50)
Body Temperature:		38.2 + 0.8	36.0 40.0 (291)

CO2 MMOL/L	22.6 + 6.8	7.0 34.0 (132)
ESR	1 + 1	0 1 (2)
FIBRINOGEN G/L	3.64 + 5.03	1.00 22.0 (18)
GGT U/L	92 + 31	19 289 (176)
LIPASE U/L	15.0 + 9.17	0.0 38.9 (56)
PROGESTERONE NMOL/L	0.239 + 0.0	0.239 0.239 (1)
TOT. T4(RIA) NMOL/L	77.4 + 23.2	47.7 125 (23)
TOT. T3(RIA) NMOL/L	3.97 + 0.0	3.97 3.97 (1)

BLOOD GLUCOSE CONCENTRATION

Equipment

- Glucometer
- Glucofilm strips
- Whole blood/or serum
- Tissue

Method

- Press button to turn meter on
- A programme number will appear in the display, ensure the number matches the number on the container of glucofilm strips
- If not press button until it does
- Open the test slide. 60 seconds will appear on the display
- Using a pipette, place a drop of blood on the pad on the reagent strip
- Press the button to start the 60 second countdown
- At 20 seconds a beep will sound
- Immediately wipe the strip with the tissue (wipe it once from bottom to top as if cleaning a kitchen knife)
- Immediately insert the strip into the slot pad side upwards and dose the slide. This must be done before the countdown reaches one second
- The test result will appear
- The result will be stored in memory unless deleted. The result will be deleted if the button is pushed while the test slide is open. It will be saved if the button is pushed while the slide is closed
- Open the slide and remove the strip

Part 3. LABORATORY EVALUATION OF THE URINARY SYSTEM (URINALYSIS).

Sample Collection.

Like any diagnostic procedure the value of a urinalysis is dependant on correct sampling technique. Cystocentesis provides the best sample as it will be free from contaminating bacteria and cells derived from the lower urinary tract. If a voided sample is to be used then it should be collected midstream. Catheterisation of the bladder is another option but haematuria is a frequent complication of this method and could interfere with the interpretation of results. Do NOT use samples collected off the floor.

Urine is not stable, especially at warm temperatures, and the urinalysis should be performed as soon as possible after collection. Casts and neutrophils disappear rapidly from hypotonic and alkaline urine, bacteria (often contaminants) will utilise glucose, and both bilirubin and urobilinogen concentrations decrease with storage or exposure to light. Refrigeration can delay the deterioration of a urine sample. Alternatively, one drop of 10% formalin added to approximately 30 mL of urine will preserve the cells and casts. This would obviously preclude bacterial culture and chemical analysis of the specimen.

If culture is to be required then the sample should be collected by cystocentesis and transferred to a sterile container.

If a urine sample can not be analysed within 30 minutes then it should be refrigerated to delay bacterial growth and preserve cells. Just prior to analysis the sample should be re-heated to room temperature.

Physical Characteristics.

- **Colour and Turbidity**

Normal urine varies from pale yellow to amber, depending on its concentration. Very dilute urine will be almost colourless. In haematuria the urine will be red and slightly cloudy, but will clear on centrifugation. The presence of haemoglobin or myoglobin urine will cause red or red/brown discolouration, while bilirubin will be dark yellow/ brown and will produce a yellow froth if the sample is shaken.

Fresh urine is usually transparent but turbidity may result from precipitation of salts on standing. Other causes of cloudy urine include cells (erythrocytes or leucocytes), crystals, mucous, bacteria, lipids, casts and sperm.

- **Odour**

The odour of urine varies between species and sex. If freshly voided urine has the odour of ammonia, it may reflect infection of the urinary tract with urea-splitting organisms.

Specific Gravity.

The specific gravity (SG) is a measure of the kidney's ability to concentrate and dilute urine, therefore providing an indication of renal function.

Specific gravity is usually determined by a refractometer on the supernatant of centrifuged urine. It is dependent on the size and number of particles (especially sodium, chloride and urea) in solution.

Assessment of renal concentrating ability can provide an early indication of renal disease is renal function will generally fail before azotaemia develops. The normal primate range should be over 1.012, usually over 1.020. Specific gravity increases due to

- Dehydration
- Diarrhoea
- Excessive sweating (fever)
- Glucosuria
- Heart failure (related to a decreased blood flow to the kidneys)
- Proteinuria
- Vomiting

Specific gravity decreases can be due to:

- Excessive water intake
- Diabetes insipidus
- Renal failure (lose of ability to resorb water)
- Glomerulonephritis and pyelonephritis.

The interpretation of routine semiquantitative urine chemistry tests can be improved by considering the results in relation to the urine SG. For example, the presence of a 2+ proteinuria at an SG of 1.010 indicates much greater protein loss than a 2+ protein at an SG of 1.035.

Urine Chemistry. (Using commercially available test strips)Use with caution, as results will vary dramatically dependent on the species being tested.

pH

This often depends on the animals diet, with more carnivorous animals have a pH from 5.5 to 7.0, and herbivores from 7.0 to 8.4. A more alkaline pH may be due to a urinary tract infection with a urease-producing bacteria. Bacterial contamination after collection can either increase or decrease the urine pH depending on the metabolism of the organism involved.

Protein

Reagent strips are most sensitive to increased albumin concentrations and will not reliably detect globulins. Positive results are graded 1+, 2+, 3+ or 4+ corresponding roughly to 0.3, 1.0, 3.0 and 10.0 g protein/L respectively. False positive tests can occur in strongly alkaline urine.

INTERPRETATION OF PROTEINURIA

- Trace proteinura may well be normal in concentrated urine (refer to urine SG).
- Proteinuria in the absence of red blood cells or leucocytes is usually of renal origin and may reflect glomerular damaged or reduced proximal tubular reabsorption (or both)
- Marked proteinuria (particularly albumin) is a feature of primary glomerular disease. Tubular diseases cause only mild to moderate proteinuria, consisting mainly of low molecular weight globulins not resorbed be defective tubular epithelial cells.
- Proteinuria (seldom >2+) accompanied by leucocytes and bacteria may reflect lower urinary tract infection
- If there is significant haemorrhage into the urinary tract, proteinuria will be marked and there will be a positive occult blood test. The blood strip is about 50x more sensitive than the protein strip therefore a positive occult blood test will always be recorded if the protein is of haemorrhagic origin.
- Haemoglobinuria and myoglobinuria will both be detected as a proteinuria and will also have a positive occult blood test.
- Proteinuria is NOT a feature of chronic renal disease unless the disease is characterised by glomerular protein leakage.

Glucose

Reagent strips impregnated with glucose oxidase react specifically with glucose producing a colour change which can be graded as: trace (5.5 mmol/L), 1+ (14.20 mmol/L), 2+ (28 mmol/L), 3+ (55 mmol/L) and 4+ (111 mmol/L)

INTERPRETATION OF GLUCOSURIA

- Glucosuria occurs whenever the blood glucose level exceeds the reabsorptive threshold of renal tubules. In most species this is approximately 10mmol/L
- Glucosuria in the abscnece of hhyperglycaemia suggests a defect in renal tubular reabsorption mechanisms – this is rare.

Ketones

Ketonuria indicates excessive fat breakdown and/or defective carbohydrate metabolism and may occur in association with diabetes mellitus or starvation.

Bilirubin

Using test strips to detect this is very insensitive.. A positive test is supportive of intravascular heamolysis and haemoglobinuria

Occult blood

Haemoglobin and myoglobin are both detected by the standard reagent strip. A positive test could therefore indicate haematuria, haemoglobinuria or myoglobinuria. With haematuria the urine is usually a cloudy red colour and clears following centrifugation

In both haemoglobinuria and myoglobinuria the urine will have a clear red to brown colour but the two can usually be differentiated by concurrent examination of a blood sample. Haemoglobinuria will not occur until plasma haptoglobin is saturated and at this stage the plasma will also be discoursed pink or red. Myoglobin however, is not bound by plasma proteins and appears in urine without discolouring the plasma. Furthermore, haemoglobinuria will most likely be associated with an anaemia while myoglobinuria will be accompanied be elevated serum muscle enzymes (CPK).

Urobilinogen

Insensitive, and of little use in animals.

URINE SEDIMENT EXAMINATION.

It is important to adopt a standard procedure fro preparing and examining urine sediment ot all valid comparison between samples. To preserve casts, crystals, cells, one drop of formalin may be added to 30ml of urine. A suggested procedure is as follows:

- Centrifuge 10mL of urine in a conical tube for 5 minutes at relatively low speeds (700G)
- Decant the supernatant (and retain for other tests eg. SG) leaving 0.5mL in which to resuspend the sediment.
- Place a drop of resuspended sediment on a slide and cover with a coverslip
- Examine by microscope under low then high power with subdued lighting and partly closed iris diaphragm.
- Identify any casts, cells or other structures using the high dry objective (40x)
- Count the number of casts, erythrocytes, leucocytes and epithelial cells in 10 representative fields and record the average number per high powered field (HPF)
- Record any crystals, bacteriafungi or other organisms.
- Stains such as new methylene blue can be added to the sediment to assist identification cells but are usually unnecessary
- Air dried smears of urine sediment can be stained routinely with Grams stain

Interpretation of the sediment examination should involve consideration of the urine SG and the method of sample collection

Erythrocytes

In normal urine there should be fewer than 5 erythrocytes per HPF. Increased numbers may be the result of trauma during collection (especially catheterisation) or an inflammatory disease somewhere in the urinary tract

Intact erythrocytes appear as small, round, slightly refractile structures of relatively uniform size. Fat droplets are similar but are usually more variable in size and float in a different plane of focus.

Leucocytes

Normal urine contains no more than 3 leucocytes per HPF. Increased numbers indicate an inflammatory condition and there might be a concurrent bacteriuria.

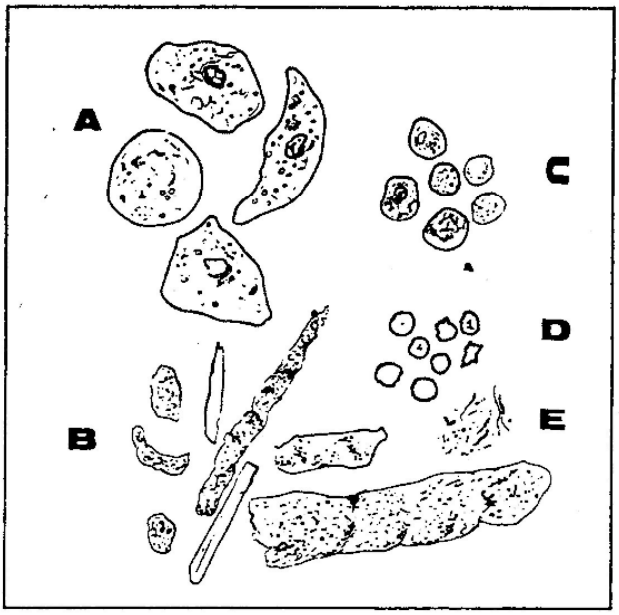
Leucocytes are slightly larger than erythrocytes and have a granular appearance. They disintegrate rapidly in alkaline or hypotonic urine

Epithelial cells

Small numbers of squamous and transitional epithelial cells are normally found in urine. Squamous cells originate from the urethra, vagina or prepuce and are highly angular cells with small central nuclei. Transitional cells can originate from the renal pelvis, ureters, bladder or proximal urethra. They are large, oval, spindle or caudate-shaped cells and may be increased in some inflammatory or neoplastic diseases

Cellular or protein derived urine deposits (approximately to scale)

A = Epithelial cells
B = various granular or hyaline casts; cellular casts are not illustrated
C = leucocytes
D = erythrocytes
E = bacteria.



Casts

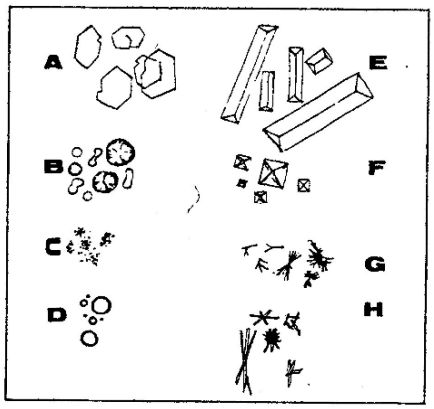
Casts are cylindrical-shaped structures composed of mucoproteins and form in the acidic environment of distal tubules. They tend to disintegrate in dilute or alkaline urine.

Fewer than 1-2 casts per low powered field (10x) are found in normal urine sediment. Increased numbers generally reflect renal disease, but the absence of casts does not exclude this possibility. Several types of casts may be recognised in urine:

- **Hyaline casts –** homogenous, translucent forms, usually associated with mild renal disease
- **Granular casts** – may contain either cellular debris or aggregates of serum protein embedded in mucoprotein and usually indicate more severe renal disease. The presence of ANY granular casts is likely to be significant.
- **Epithelial or cellular casts –** contain desquamated tubular epithelial cells and/or leucocytes
- **Waxy Casts** – broad, nonregular structures with broken or square rods. They are derived from degenerating cellular and granular casts in chronic tubular infections
- **Fatty casts –** Contain fat globules derived from degenerating tubular epithelial cells.

Crystals
Some types of crystals may have pathogenic significance but others are found normally in the urine of some species.

Miscellaneous structures
A variety of organisms including bacteria, fungi and even parasite eggs can be found in urine and may or may not have pathological significance. The presence of bacteria or fungi may indicate contamination of the specimen rather than urinary tract infection. If such organisms are present in association with increased numbers of inflammatory cells then they may be significant and culture is recommended.



Crystalline or non cellular urine deposits. (size approximate)

A = cystine
B = carbonates
C = amorphous urates or phosphates
D = fat globules
E = triple phosphates (ammonium magnesium phosphate)
F = oxalate
G = bilirubin (brown/orange colour)
H = bi urates or stellar phosphates.

APPENDIX

Materials Required for Unopette Procedure

The Unopette procedure consists of a disposable diluting pipette system that provides a convenient, precise, and accurate method for obtaining a red blood cell count. To perform a red blood cell count using the Unopette method, you will need to obtain the following materials:

· A disposable Unopette (see fig. 7-8) for RBC counts. The Unopette consists of

-a shielded capillary pipette (10 microliter (Fl) capacity), and
-a plastic reservoir containing a premeasured volume of diluent (1:200 dilution).
· Hemacytometer and coverglass
· Microscope with light source

· Hand-held counter
· Laboratory chit

Unopette Procedure

The Unopette procedure for counting red blood cells is as follows:

1. Puncture the diaphragm in the neck of the diluent reservoir with the tip of the capillary shield on the capillary pipette. See figure 7-9.

2. After obtaining free-flowing blood from a lancet puncture of the finger, remove the protective plastic shield from the capillary pipette. Holding the capillary pipette slightly above the horizontal, touch the tip to the blood source (see fig. 7-10, view A). The pipette will

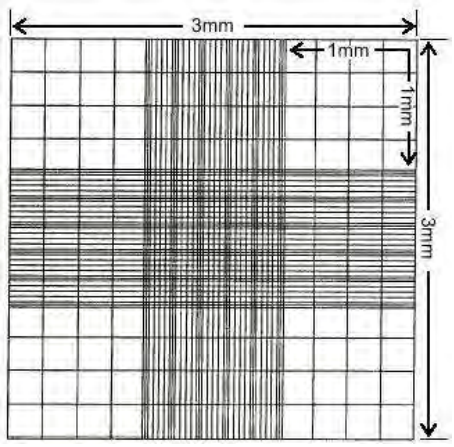


Figure -Improved Neubauer Ruling.

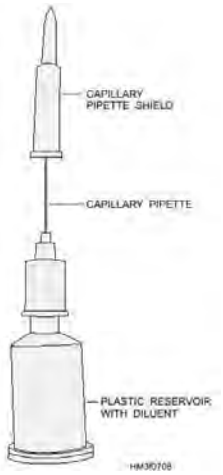


Figure .-Unopette(r) for RBC count.

fill by capillary action. When blood reaches the end of the capillary bore in the neck of the pipette, filling is complete and will stop automatically. The amount of blood collected by the capillary tube is 10 Fl. Wipe any blood off the outside of the capillary tube, making sure no blood is removed from inside the capillary pipette. (An alternative source of blood is a thoroughly mixed fresh venous blood sample obtained by venipuncture. See figure 7-10, view B.)

3. With one hand, gently squeeze the reservoir to force some air out, but do not expel any diluent (fig. 7-11). Maintain pressure on the reservoir. With the other hand, cover the upper opening of the capillary overflow chamber with your index finger and seat the capillary pipette holder in the reservoir neck (see fig. 7-11).

4. Release pressure on the reservoir and remove your finger from the overflow chamber opening. Suction will draw the blood into the diluent in the reservoir.

5. Squeeze the reservoir gently two or three times to rinse the capillary tube, forcing diluent into but not out of the overflow chamber, releasing pressure each time to return diluent to the reservoir. Close the upper opening with your index finger and invert the unit several times to mix the blood sample and the diluent. See figure 7-12.

6. For specimen storage, cover the overflow chamber of the capillary tube with the capillary shield.

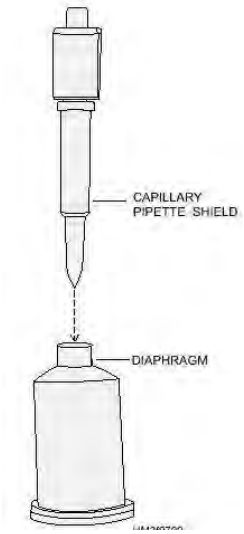


Figure .-Puncturing the diaphragm of diluent with the capillary pipette shield.

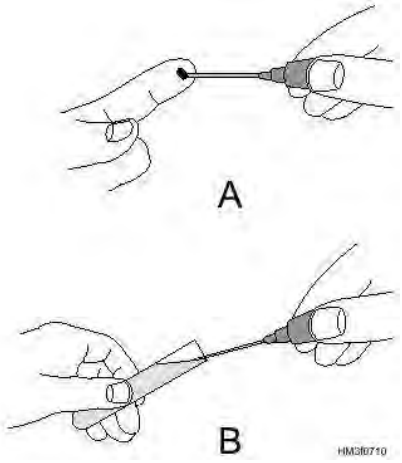


Figure.-Drawing blood into the Unopette capillary tube: A. From a finger puncture; B. From a venous blood sample.

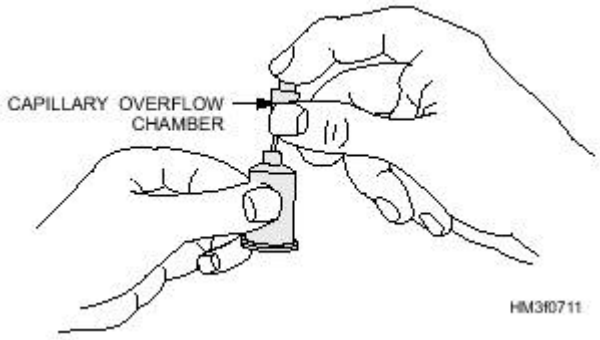


Figure .-Preparing reservoir to receive blood from the capillary tube.

7. Immediately prior to cell counting, mix again by gentle inversion, taking care to cover the upper opening of the overflow chamber with your index finger.

8. Place the coverglass on the hemacytometer counting chamber, making sure coverglass is clean and free of grease. (Fingerprints must be completely removed.)

9. Remove the pipette from the reservoir. Squeeze the reservoir and reseat the pipette in the reverse position, releasing pressure to draw any fluid in the capillary tube into the reservoir. Invert and fill the capillary pipette by gentle pressure on the reservoir. After discarding the first 3 drops, load (charge) the counting chamber of the hemacytometer by gently squeezing the reservoir while touching the tip of the pipette against the edge of the coverglass and the surface of the counting chamber (fig. 7-13). A properly loaded counting chamber should have a thin, even film of fluid under the coverglass (fig. 7-14, view A). Allow 3 minutes for cells to settle. If fluid flows into the grooves (moats) at the edges of the chamber or if air bubbles are seen in the field, the chamber is flooded and yes"> must be cleaned with distilled water, dried with lens tissue, and reloaded (fig. 7-14, view B). If the chamber is underloaded, carefully add additional fluid until properly loaded.

10. Place the loaded hemacytometer into a petri dish with a piece of dampened tissue to keep the hemacytometer from drying out (fig. 7-15). Allow 5 to 10 minutes for the cells to settle.

11. Once the cells have settled, place the hemocytometer on the microscope. Use the low-power lens to locate the five small fields (1, 2, 3, 4, and 5) in the large center square bounded by the double or triple lines. See figure 7-16. Each field measures 1/25 mm 2 , 1/10 mm in depth, and is divided into 16 smaller squares. These smaller squares form a grid that makes accurate counting possible.

12. Switch to the high-power lens and count the number of cells in field 1. Move the hemacytometer until field 2 is in focus and repeat the counting procedure. Continue until the cells in all five fields have been counted. Note the fields are numbered clockwise around the chamber, with field 5 being in the center.

Figure.-Mixing blood sample and diluent.

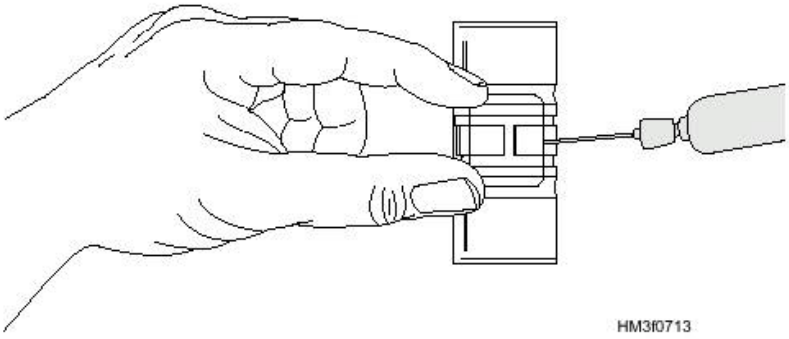


Figure.-Loading the counting chamber.

Count the fields in this order. To count the cells in each field, start in the upper left small square and follow the pattern indicated by the arrow in field 1 of figure 7-16. Count all of the cells within each square, including cells touching the lines at the top and on the left. **Do not count any cells that touch the lines on the right or at the bottom.**

13. Total the number of cells counted in all five fields and multiply by 10,000 to arrive at the number of red cells per cubic millimeter of blood.

Example: Total number of cells counted = 423.	
Multiply:	
423 x 10,000	= 4,230,000
Total red cell count	= **4,2300,000 cells/mm^3**

NOTE: The number of cells counted in each field should not vary by more than 20. A greater variation may indicate poor distribution of the cells in the fluid, resulting in an inaccurate count. If this happens, the test must be repeated.

3.20 PROTOCOL FOR WRITING MEDICAL RECORDS

W.Boardman, S.Unwin

Medical records should be written up as soon as possible after a procedure has taken place.
A systematic and consistent approach is important when writing medical records. The headings below act as a template for writing records – the S.O.A.P format

S: Subjective information that you are told about the case. For example:
History or Hx:

A brief account of the recent history of the animal should be recorded including demeanour, duration of problem, others affected, clinical signs, recent medication.

Reports or Rpt:

Report from caregiver regarding the problem.
Updates regarding changes in the condition, effectiveness and acceptance of treatment.

O: Objective information that you as the veterinarian observes, or procedures you conduct. For example:
Observations or Obs:

Brief description of the observation made by the veterinarian.

Procedures or Proc:

List all procedures carried out on the animal including:

Anaesthesia or manual restraint

Reason for anaesthesia i.e. what is the reason for the anaesthetic e.g. examination to determine cause of illness/injury, quarantine procedures, treatment, transportation etc.
Anaesthetic procedure

- Fill in anaesthesia form in detail and add notes only in the medical notes
- Pre-medicants used: dose, time and route
- Immobilisation drugs including top ups: dose, time and route
- Antagonists: dose, time and route
- Recovery times
- Rating of anaesthesia including emergencies.

Full clinical examination
Including temperature, pulse, respiratory rates and weight

Describe any abnormalities and explain which systems have been examined and which have not.

Diagnostic procedures

Radiography, MRI, ultrasound, gastric/bronchial lavage etc

Other procedures

Samples taken e.g. blood including which vein (B/S), faeces, urine – how taken, swabs- where, how, skin scrapings and the tests to be performed and where.

Tuberculosis test: type of test used for screening i.e. intra dermal injection, site used intra dermal injection, concentration and dose tuberculin, batch no and manufacturer of the tuberculin.

Vaccinations: type of vaccine, batch no and manufacturer, route and dose

Surgery – S_x: description of surgical procedure including suture material

Contraceptive: type of contraceptive device

A: Analysis or assessment. This is where you place your differential diagnosis, and analyse the situation, including any results from testing

- **This is the opportunity to explain/ commentate/ speculate on what's occurring with the case. This should be done updated regularly.**

- Include list of differential diagnoses DD_X and diagnosis D_X if known.

- Results of any tests performed including TB results.
- Information should be written up as soon as possible.

P: Plan – what you intend to do. This must always include a follow up – either signing off a problem, or a follow up visit. For example:

- **Plan for future workups, alternate treatment ideas if present one is not effective, rechecks, bandage changes, or other actions to be taken.**
- Dates for rechecks, reweighing etc.

Treatments or T_X

Treatments should be recorded in the following way
Drug → generic, tradename, formulation and concentration
Dosage → amount, route, frequency, duration, start and finish date

Eg: Baytril inj (Enrofloxacin 50mg/ml). 2ml (100mg) IV

Prescription or R_X

- Drugs prescribed should be recorded in the following way
- Drug → tradename, generic name, formulation and concentration
- Dosage → amount, route, frequency, duration, start and finish date

- Eg: Clavulox 250mg (Clavulanic acid + amoxycillin).

- 1 tab PO BID for 7 days. Start 21/09/01. Finish 27/09/01

STANDARD ABBREVIATIONS THAT CAN BE USED IN MEDICAL RECORDS

Abbrev	**Meaning**	**Abbrev**	**Meaning**
ad lib	at pleasure	biochem	biochemistry
a.c	before meals	Assess	assessment
a.d.	right ear	Bacto	bacteriology
a.s	left ear	bx	biopsy
a.u	both ears	C/S	culture and sensitivity
adm	administer	cyto	cytology
aq	water	DD_x	differential diagnosis
bid	twice daily	Diag	diagnosis
c	with	D_x	diagnosis
cap	capsule	EUA	examination under anaesthesia
disp	dispense	Exam	Examination
gtt	drop	Hx	history
h	hour	IC	intracardiac
IM	intramuscularly	ICe	Intracoelomic
inj	inject	LF	left fore
IP	intraperitoneally	LH	left hind
IV	intravenously	NAD	no abnormalities detected
o.d	right eye	NSF	no significant findings
o.s	left eye	N/E	not examined
o.u	both eyes	O/N	overnight
p.c	after meals	Obs	observation
po	per os (by mouth)	Path	pathology
prn	as needed	Proc	procedure
q	every	Quar	quarantine
q24h	every day	Re exam	re examination
q4h	every 4 hours	RF	right fore
qd	every day	RH	right hind
qid	four times per day	Rpt	report
qod	every other day	R_x	prescription
qs	A sufficient quantity	S_x	surgery
SC or SQ	subcutaneously	T_x	treatment
sig	instructions for patient	UA	urinanalysis
Stat	immediately	Vacc	vaccination
Sus	suspension	w/e	weekend

p			
tid	three times daily		
ut dict	as directed		

Also used is a system of explaining the length of the treatment:

5/7 means for five days (out of a week)
1/12 means for 1 month (out of a year)
1/52 means for 1 week (out of a year)

3.21 SURGERY BASICS

Lesa Longley MA BVM&S DZooMed (Mammalian) MRCVS. Reviewed, Steve Unwin

Introduction

The veterinary clinician may be called upon to perform surgery on animals in their care. This section is a basic introduction to surgery, in particular suture materials and patterns. Clinicians should take every opportunity to practice these techniques. For example, taking the opportunity to practice surgical approaches when conducting a post mortem.

Reasons for surgery

- Trauma

This is often due to con-specific aggression within groups of primates, but in rescue centres may also be anthropogenic (for example traps or bullet wounds).

- Reproductive

This usually relates to contraception – such as implants in females and vasectomy or castration in males. Caesareans may also be performed.

- Dentistry

This may be minor—such as descaling calculus—or more involved—such as tooth extraction or root canal fillings.

Suture material & pattern selection

Various types of suture are available. Consideration should be given to the requirements of the material, i.e. the purpose of utilizing it.

- Absorbable vs. non-absorbable

Advantages of **absorbable** suture material include breakdown by the body and thereby no foreign body is left. The main disadvantage is a variation in the period of wound support.

Examples of absorbable suture material: catgut, poliglecaprone 25 (Monocryl®), polyglactin 910 (Vicryl®), and polydioxanone (PDS®). Short-term wound support is provided by catgut, Monocryl® or Vicryl Rapide®; medium term support by Vicryl®, Dexon® or Biosyn®; and long-term support by PDS® or Maxon®.

Non-absorbable suture material provides permanent support to wounds. However, foreign material is left in the body and a suture sinus or extrusion of suture may occur.

Examples of nonabsorbable materials: silk (Mersilk®), nylon (Ethilon®, Nurolon®), polypropylene (Prolene®), and stainless steel.

- Monofilament vs. multifilament

There are many advantages to **monofilament** suture material – it has a smooth surface, low friction means less drag and less tissue trauma, no bacteria may be harboured, and there is no capillarity (i.e. no 'wicking' effect). However handling and knotting are more difficult than multifilament material, burial of sutures ends and knots can also be problematic with monofilament, and it is more prone to stretching.

Multifilament material tends to be stronger, and is soft and pliable with good handling. Bacteria may be harboured within multifilament suture, it is inclined to wicking (which allows bacteria to migrate into deeper tissues), and tissue trauma may result due to 'drag' from the material and a cutting effect.

- Biological vs. synthetic

Biological suture materials have excellent handling and knotting capabilities, are economical, and are absorbed by hydrolysis. They are absorbed by enzymatic action, may cause tissue reactions, and have an unpredictable rate of absorption.

Synthetic materials resemble natural substances, but have predictable absorption and are strong.

- Packaging

Foil pack. In general—though not always—suture in these packs has a swaged-on needle.

Reel – the suture material is stored in a preservative such as alcohol. An assistant removes the cap and pulls the end of the suture material, allowing the surgeon to grasp a sterile region. Pull the suture upwards—ensuring not to touch the non-sterile edges of the cap—before cutting the required length. Use a sterile needle to attach the suture.

Absorbable sutures

- Catgut

This is obtained from sheep intestinal submucosa or cattle intestinal serosa. It is absorbed by phagocytosis and enzymatic degradation, and there is a large inflammatory response. The rate of absorption depends on the site and wound conditions.

Catgut loses tensile strength rapidly and unpredictably. Chromic gut is better than plain gut – chromic gut has reduced inflammation associated and an extended tensile strength (50% by 14 days, 0% by 28 days). Catgut has a tendency to swell and weaken when wet. It is also weakened by knotting, but ties good ligatures (you shouldn't need to use a surgeon's knot). There is some controversy over the use of catgut due to the risk of TSE (transmissible spongiform encephalopathy). Only use for ligating.

- Poliglecaprone 25 (Monocryl®)

This is a synthetic monofilament suture that is absorbed by hydrolysis. It is virtually memory free (i.e. doesn't return to its previous shape after deformation) – meaning that it handles well and knots securely. This material has the highest

tensile strength of any monofilament absorbable suture – with 60% retained at 7 days, 30% at 14 days, and gone by 90-120 days.

- Polyglactin 910 (Vicryl®, Vicryl Rapide®)

This is a braided synthetic absorbable suture. It is coated to reduce tissue drag and improve knotting characteristics. It is absorbed by hydrolysis and therefore has a predictable loss of tensile strength – 55% retained at 14 days, 40% at 21 days, 10% at 28 days, and gone by 56-70 days.

The initial tensile strength of Vicryl Rapide® is 70% that of Vicryl®; Vicryl Rapide® retains 50% at 5 days, 0% at 14 days, and is gone by 42 days. It is usually used in skin (e.g. intradermal sutures), mucosa (where healing is rapid), or for fractious animals (again using intradermal skin sutures).

- Polyglycolic acid (Dexon®, Dexon II®, Safil®)

This is a braided synthetic multifilament polymer—usually coated—with high tissue drag and poor knot security. It is broken down by hydrolysis, the products of which are bacteriostatic *in vivo* – with 67% strength retained at 7 days, 35% at 21 days, and gone by 60-90 days.

- Polydioxanone (PDS®, PDSII®)

This suture material is a monofilament synthetic polymer. It has low tissue drag. Degradation is by hydrolysis, but at a slow rate to provide extended wound support – 75% is retained at 14 days, 50% at 28 days, 25% at 42 days, and gone by 180 days. It is useful for slowly healing tissues such as tendon and fascia.

- Polyglyconate (Maxon®)

This synthetic monofilament has similar properties to PDS®.

- Panacryl®

This braided synthetic absorbable suture retains 80% tensile strength at 3 months and 60% at 6 months. Thus it provides extended wound support.

Non-absorbable sutures – these are often used for repair of tendons or hernias

- Silk (Mersilk®, Silkam®)

This braided multifilament is usually coated to decrease capillarity. Ultimately it is absorbed, but extremely slowly – no tensile strength remains at 12 months. There is significant tissue reaction. Silk has nice handling characteristics but poor knot security. Its main use is for ligatures, but it should never be used in the presence of infection or contamination. Silk is not recommended for use in sanctuaries for these reasons.

- Nylon (Ethilon®, Neurolon®, Dermalon®)

This is usually monofilament, but multifilament nylon is available. This has a high tensile strength – losing 10-20% per year. Nylon has a high 'memory', resulting in poor handling and knot security. Its main use is for skin sutures (which have to be removed after healing has occurred).

- Polypropylene (Prolene®, Premilene®, Fluorofil®)

This is a monofilament polymer. High memory and poor handling mean that good knots are difficult to tie, but with careful tying strands flatten at the knot to enhance holding. This material is virtually inert in tissues, and is used in meshes to repair large tissue defects.

- Stainless steel

This may be either monofilament or braided. It has high tensile strength and good knot security. However it has poor handling characteristics and breaks if subjected to cyclic loading (i.e. repeated stresses). The main uses for stainless steel are in orthopaedic surgery, and as haemostatic clips and skin staples.

Suture selection

- Sutures are no longer needed when a wound reaches maximal strength. Use non-absorbable materials or those with extended absorption for tissues that heal slowly, such as tendon.
- Foreign bodies in potentially contaminated tissues may convert contamination to infection. Therefore use monofilament or absorbable suture in potentially contaminated tissues.
- Where cosmetic results are important, close and prolonged apposition of wounds and avoidance of irritants will produce the best result. Use the smallest inert monofilament suture. Close subcuticularly where possible. Topical skin glue may be useful.
- Use rapidly absorbed sutures in the urinary and biliary tracts, or else you risk the suture becoming a nidus for stone formation.

Suture size is recorded as either Metric (Eur.Ph.) or Imperial gauge (USP). Metric measurements are in tenths of a mm, from 0.1 to 10. Imperial measurements range from 11/0 to 6 (although catgut is different!) For orthopaedic wire, the measurement is a B&S wire gauge, in mm.

Choose the smallest size of suture for the natural strength of the tissue. Reinforce with retention sutures if there may be sudden strains on the suture line post-operatively.

Surgical needles come in a variety of sizes, shapes and types. The needle should pass through the tissue without excessive force and with minimal disruption of tissue architecture. Swaged needles—that produce less tissue trauma but are more expensive—are preferred to closed eye needles—that require threading and pull a double strand of suture through the tissue. Curved needles are easier to use with instruments.

Needle shapes: Conventional cutting needles have the apex of the edges on the inside curvature. Reverse cutting needles have the apex of the edges on the outside curvature. Taper point needles separate tissue but do not cut. **PHOTOS

Ligatures must be secure! Avoid granny knots and half-hitch or tumbled knots, which will slip. (See references)

- Simple knot
- Square knot – one hand or two hands

- Surgeon's or friction knot
- Deep tie – ensure this is a square knot
- Ligation around a haemostatic clamp
- Instrument tie
- Transfixing ligature

Suture patterns (see references)

- Interrupted
  - Simple interrupted
  - Cruciate
  - Horizontal or vertical mattress: this is a tension-relieving pattern
- Continuous
  - Simple continuous: including intradermal pattern (finishing with a surgeon's or Aberdeen knot)
  - Intradermal

For interrupted patterns, 4 throws should be used on knots. For continuous patterns, use 5 throws at the start and 6 at the end (as the end knot tends to be less secure and therefore needs an extra throw).

Surgical instruments **PICTURES

As basics for suturing, you need scissors, forceps and needle holders. Other instruments are required for more involved surgery – for example haemostatic clamps, Allis tissue forceps, dental elevators, retractors, and towel clamps.

Common procedures

Trauma

E.g. digit/tail amputations after fighting, attack wounds, trap or gunshot wounds. Not all fight wounds require surgery, and many will be infected so primary closure will not be possible.

Primate wounds usually heal rapidly, even particularly severe fight wounds. In many cases, surgical intervention is not necessary – and may even be contraindicated if infection is present (as wound dehiscence is likely). Veterinary experience will determine when surgery is required and when it is not.

Reproductive

E.g. Contraceptive implant, caesarean section, castration, vasectomy

NB It is important to use an intradermal pattern in the skin of primates to prevent self-trauma post-operatively.

Dental

E.g. Extractions

Further information

Fossum, T.W. (2006) Small Animal Surgery, 3rd Edn. Mosby
Niles, J. & Williams J. (1999) Suture materials and patterns. *In Practice*. **21** (6): 308–320

http://www.ethicon.com/ - Suture materials
http://www.animalcare.co.uk/Instruments-Equipment/default.aspx - Surgical instruments
http://cal.vet.upenn.edu/projects/surgery/5000.htm (provides suture pattern videos for practice)

SECTION 4

DISEASE CONDITIONS

SECTION 4

DISEASE CONDITIONS

Disease issues important to sanctuaries

4.1 AN OVERVIEW OF SELECTED DISEASES OF AFRICAN PRIMATES

Wayne Boardman, Owen Slater and Steve Unwin.

An indication of high, medium or low concern is provided next to each disease, based on either published data or anecdotal evidence. Disease is of higher concern if it has relatively high mortality/ morbidity, if it is Zoonotic and/ or if it would have as yet unknown consequences, especially for release programmes.

VIRUSES

SIMIAN VARICELLA VIRUS (Moderate Concern)

Aetiology: Cercopithicine herpes virus 6, 7 and 9. Group of closely-related herpesviruses including Delta herpesvirus, Medical Lake macaque virus, Liverpool vervet monkey virus, and others. All are antigenically related to human varicella-zoster.

Transmission: Respiratory. Latency is common and the origin of some outbreaks is unexplained.

Clinical: Affects patas, African green monkeys, macaques, chimps and gorillas. Herpetic rash, depression, respiratory difficulty.

Diagnosis: Usually based on clinical signs. Virus isolation, and PCR

Pathology: Vesicles on skin, oral mucous membranes, and esophagus; focal necrosis in lung, liver, spleen, lymph nodes, adrenal, bone marrow, intestinal tract. Multinucleated giant cells and intranuclear inclusion bodies. Becomes latent in ganglia.

HERPES SIMPLEX VIRUS (Low Concern)

Aetiology: Herpes simplex virus (type 1 and 2)

Transmission: Latent or active infection in many humans, which are the natural reservoir. Human to monkey and monkey to monkey transmission from active lesions.

Clinical: Lesions may be local or generalized. Oral vesicles and ulcers, conjunctivitis, encephalitis, death. Chimpanzees, gorillas, and gibbons can be infected, but usually remains confined to skin, oral cavity, external genitalia, and conjunctiva.

Diagnosis: Usually based on clinical signs. Virus isolation, electron microscopy –or florescent antibody can confirm. Commercial kits available.

Pathology: Oral, lingual, labial, or genital vesicles & ulcers. Conjunctivitis, keratitis. Multinucleated giant cells and intranuclear inclusion bodies.

AFRICAN MONKEY HERPES VIRUS (Low Concern)

Aetiology: Simian Agent 8 (SA8) (a.k.a. *Herpesvirus papionis*)

Transmission: Saliva and other bodily fluids.

Clinical: Usually asymptomatic in baboons. Genital and oral vesicles and pustules with genital lesions becoming more severe. Latency and recrudescence can occur. Has not been described in great apes.

Diagnosis: As for HSV 1 and 2.

Pathology: As for HSV 1 and 2. Multinucleated giant cells and intranuclear inclusion bodies.

CYTOMEGALOVIRUS (Moderate concern)

Aetiology: Betaherpesvirus

Transmission: Horizontal (shed in urine, saliva, blood), transplacental, highly species-specific and may become latent in glandular tissue

Clinical: Usually asymptomatic. Chimpanzees and gorillas have been infected with clinical signs of diarrhoea, anorexia and lethargy. Widespread latent infections in macaques, with most seroconverting during the first year of life. Disease produced only in foetuses and immunodeficient individuals. CNS and respiratory tract signs. CMV is a common opportunistic infection in SIV and SRV infected macaques.

Diagnosis: Usually based on clinical signs. Confirmed based on histopathology of discrete small foci of rounded cells, fluorescent antibody or enzyme immunoassay.

Pathology: In immunodeficient animals, generalized infections with necrotizing meningitis and neuritis, interstitial pneumonia, arteritis, enterocolitis, orchitis, and focal necrosis in liver and spleen. Characteristic large basophilic intranuclear inclusion bodies and granular eosinophilic cytoplasmic inclusion bodies in mesenchymal cells (not surface epithelium like other herpesviruses).

EPSTEIN-BARR VIRUS (Moderate concern)

Aetiology: Nonhuman primate EBV-related Herpesviruses

Transmission: Prolonged contact with saliva

Clinical: Most infections are asymptomatic and latent. Specific chimpanzee, gorilla and orangutan types. In immunodeficient animals, EBV has been associated with lymphoma and with squamous epithelial proliferative lesions. Most humans are subclinical carriers of the human type.

Diagnosis: Serology

Pathology: Extranodal B-cell lymphoma or squamous cell proliferations resembling oral hairy leukoplakia on oral, genital, and cutaneous surfaces in immunodeficient animals. Intranuclear inclusions are present in epithelial lesions.

ENCEPHALOMYOCARDITIS VIRUS (High Concern)

Aetiology: Encephalomyocarditis virus (Family: Picornaviridae, Genus: Cardiovirus)

Transmission: Oral, other routes suspected. Probable rodent reservoir with contamination of food and surfaces.

Clinical: Sudden death. Causes myocarditis in nonhuman primates, pigs, elephants, some others. EMCV is probably not a significant human pathogen, although some people are seropositive.

Diagnosis: Virus isolation especially in acute cases and serology.

Pathology: Pericardial effusion, pale areas in myocardium. Myofiber necrosis with inflammation and oedema. Secondary lesions of acute heart failure. Extensive myocardial scarring in animals that survive acute infection. Some strains of EMCV cause necrosis of the exocrine pancreas in some species.

MONKEYPOX (Moderate concern)

Aetiology: Orthopoxvirus immunologically related to smallpox and vaccinia

Transmission: Zoonotic disease of monkeys and humans in tropical rain forests of western and central Africa. Spread via aerosols, biting and other contact. Animal reservoir unknown, but possibly squirrels and probably not monkeys. Occurs sporadically.

Clinical: Vaccine is protective, but hasn't been used since 1980. Disease in children resembles discrete ordinary smallpox, except lymphadenopathy occurs commonly in monkeypox. Human to human transmission has occurred. In monkeys (red tail monkey, Allen's swamp monkey, Lesser white-nosed monkey, red colobus), disease may be mild to fatal. Usually see 1 to 4 mm diameter cutaneous papules that become pustules and then crust over and drops off, leaving small scars. In more severe disease, facial oedema, dyspnoea, oral ulcers, and lymphadenopathy.

Diagnosis: Virus isolation, Electron microscopy (vesicular fluid superior to swabs, and serology.

Pathology: Hyperplasia and necrosis of epidermis, with swelling of keratinocytes and large eosinophilic intracytoplasmic inclusions. Visceral lesions can occur.

YABA POX (Low concern)

Aetiology: Unclassified poxvirus closely related to swinepox

Transmission: Mosquito vector.

Clinical: Natural infections have occurred in macaques and baboons. Humans are also susceptible. Rapidly growing subcutaneous nodules up to 4 cm diameter on head and limbs. These spontaneously slough and heal in 6 to 12 weeks.

Diagnosis: Tissue culture, immunohistochemistry and serology

Pathology: Unlike other poxviruses, Yaba pox infects histiocytes rather than epithelial cells. Yaba pox virus induces subcutaneous proliferation of round to polygonal histiocytes which often contain eosinophilic cytoplasmic inclusions. Usually described as benign histiocytomas. Similar to lumpy skin disease of cattle.

MOLLUSCUM CONTAGIOSUM (Low concern)

Aetiology: poxvirus unrelated to smallpox

Transmission: Unknown

Clinical: Humans, chimpanzees and macaques. Smooth-surfaced, hemispheric, waxy, umbilicated epithelial papules, 3-8 mm diameter, anywhere on skin, but especially eyelid and groin.

Diagnosis: Tissue culture or serology

Pathology: Marked acanthosis with large basophilic intracytoplasmic inclusion bodies that become more prominent towards the skin surface.

MEASLES (High concern)

Aetiology: Human measles virus (Paramyxoviridae: Morbillivirus)

Transmission: Respiratory. Human reservoir. Measles is not a natural disease of macaques, but is acquired through contact with humans.

Clinical: Affects apes, macaques, baboons, African green monkeys, colobus, . May be subclinical or cause maculopapular rash, conjunctivitis, facial erythema, respiratory difficulty, diarrhoea. Causes temporary immunosuppression.

Diagnosis: Tissue culture or serology

Pathology: Focal necrosis on oral mucous membranes, interstitial pneumonia, syncytial cells in skin, lymph nodes, lung. Intranuclear and intracytoplasmic inclusion bodies.

ADENOVIRUS (Moderate concern)

Aetiology: Adenovirus (> 80 species)

Transmission: Spread through respiratory, faecal-oral and direct contact modes.

Clinical: Usually asymptomatic. Conjunctivitis and respiratory infections, diarrhoea, pancreatitis. Severe infections in immunodeficient animals. Chimpanzees appear more susceptible to disease than other great ape species.

Diagnosis: Virus isolation or serology (complement fixation). Electron microscopy not that reliable.

Pathology: Frequently isolated from intestine and lung of healthy animals. Necrotizing alveolitis and bronchiolitis, pneumonia, necrotizing pancreatitis, enteritis. Intranuclear inclusions vary from small and eosinophilic to large, basophilic, and "smudgy".

RESPIRATORY SYNCYTIAL VIRUS (Moderate concern)

Aetiology: RSV - Family: Paramyxoviridae

Transmission: Aerosols.

Clinical: Chimpanzees affected with clinical signs of coughing, sneezing, mucopurulent nasal discharge.

Diagnosis: Tissue culture (syncytial cell formation) and serology

Pathology: Bronchopneumonia with synctial cell formation**.**

HUMAN METAPNEUMOVIRUS (High concern)

Aetiology: In the Paramyxoviridae family closely related to RSV

Transmission: Aerosols, possibly faecal-oral as virus can be found in faeces several days after resolution of clinical signs.

Clinical: Chimpanzees affected with clinical signs ranging from mild coughing, sneezing, to thick mucopurulent nasal discharge, lethargy, dyspnoea abortion and death. Gorillas appear to be largely asymptomatic. Also reported in macaques.

Diagnosis: Tissue culture, PCR (faecal and respiratory secretions), and serology

Pathology: Bronchointerstitial pneumonia**.**

POLIOMYELITIS (Moderate concern)

Aetiology: Poliovirus. Enterovirus in the Family Picornaviridae.

Transmission: Faecal-oral with many asymptomatic carriers.

Clinical: Chimpanzees, bonobos, gorillas and colobus are susceptible. Symptoms range from fever, diarrhoea, nuchal rigidity to paralytic disease.

Diagnosis: Tissue culture, PCR, immunofluorescence and serology

Pathology: Non-purulent myeloencephalitis. Extensive loss of ganglia cells and marked glial cell proliferation with preferential sites in the spinal cord, cerebella nuclei and diencephalon.

PAPILLOMAVIRUS (Low concern)

Aetiology: Papillomavirus

Transmission: Direct skin contact

Clinical: Papillomas on skin, oral or genital mucosa.

Diagnosis: Papillomavirus antigens can be demonstrated by immunohistochemistry and virions by electron microscopy.

Pathology: Focal hyperkeratosis, parakeratosis, acanthosis.

FOCAL EPITHELIAL HYPERPLASIA OF CHIMPANZEES (Low concern)

Aetiology: Papovavirus

Transmission: Direct contact

Clinical: Circumscribed soft elevations of the oral mucosa of lips, tongue, gingiva. This is usually a benign condition that may persist for years or may spontaneously regress.

Diagnosis: Papovavirus like particles can be demonstrated in nuclei of epithelial cells. PCR and transmission electron microscopy.

Pathology: Focal acanthosis with koilocytosis, mild chronic inflammation.

SV40 (Low concern)

Aetiology: Papovavirus (Polyomavirus subgroup)

Transmission: Respiratory. Virus is shed in urine.

Clinical: Usually none. Widespread latent infection in wild and captive macaques. In immunodeficient animals can cause CNS and respiratory signs.

Diagnosis: PCR

Pathology: Usually none. In immunocompromised animals, interstitial pneumonia, renal tubular necrosis, encephalitis, demyelination (progressive multifocal leukoencephalopathy). PML probably represents a reactivated latent infection, whereas pneumonia, nephritis, and meningeoencephalitis. Lesions in the brain may have typical distribution of PML or may be around ventricles (particularly in brainstem) and in superficial cortex. Astrocytes and oligodendrocytes are infected. Large basophilic intranuclear inclusions in lung, oligodendroglia, renal tubular epithelium.

YELLOW FEVER (Low concern)

Aetiology: Arbovirus (Flaviviridae)

Transmission: Via mosquitoes (numerous *Aedes* species)

Clinical: Usually none in old world monkeys. The exception is Galago (*Galago crassicaudatus*) with mortality rates up to 50% in laboratory infected animals

Diagnosis: Virus isolation, serology, RT-PCR

Pathology: Fatty liver degeneration, extensive hepatocellular necrosis, and hemorrhagic diathesis.

SIMIAN HEMORRHAGIC FEVER VIRUS (Moderate concern in species affected)

Aetiology: Togaviridae, Arterivirus

Transmission: Simian Hemorrhagic Fever Virus is endemic in some wild Patas monkeys (*Erythrocebus patas*) and possibly other African species (African green monkeys, baboons), which remain persistently viraemic, but asymptomatic for life. Animals may be viraemic without antibody production. Transmission from Patas to macaques in laboratory settings appears to require parenteral exposure to blood or body fluids. The virus spreads much more readily among macaques by contact or aerosol.

Clinical: None in African primates. The virus causes explosive epidemics with nearly 100% mortality in captive macaques. Clinical signs in macaques include fever, anorexia, depression, facial oedema, epistaxis, cutaneous and subcutaneous haemorrhage. Severely elevated LDH, disseminated intravascular coagulation, thrombocytopenia.

Any epizootic of hemorrhagic disease should be reported to the special pathogens branch, Centres for Disease Control, Atlanta, GA.

Diagnosis: Virus isolation, indirect immunofluorescence, serology

Pathology: None in African primates. In Macaques gross lesions are variable, may be absent, and are seen only in the final stage of disease. Petechial haemorrhage on mucosal and serosal surfaces, haemorrhage and necrosis of the mucosa of the proximal duodenum, splenomegaly, splenic lymphoid follicles ringed with a zone of bright red haemorrhage. Microscopic changes consist of lymphoid necrosis, vasculitis, haemorrhage, and intravascular fibrin deposition (DIC). Large amounts of fibrin are present in splenic cords. Lymphohistiocytic meningoencephalitis occasionally present. Hepatic necrosis with Councilman's bodies is not a feature of simian hemorrhagic fever, unlike other hemorrhagic fevers. Additionally, in SHF aspartate aminotransferase>alanine aminotransferase, while the reverse is true in the other hemorrhagic fevers.

HEPATITIS A VIRUS (Low concern)

Aetiology: Picornaviridae (Hepatovirus or Enterovirus)

Transmission: faecal-oral

Clinical: Infects humans, chimpanzees, African green monkeys. Seroconversion and elevation of transaminases are usually the only clinical evidence of infection. Some HAV isolates may be unique to nonhuman primates. Zoonotic potential. A vaccine (Havrix) is available and should be required for all humans working with chimpanzees.

Diagnosis: Virus isolation is not practical. Serology (ELISA, CF, RIA)

Pathology: Periportal and parenchymal mononuclear inflammation, slight focal hepatocellular degeneration and necrosis with acidophilic bodies, Kupfer cell hyperplasia. Chimpanzee pathology shows evidence of bile duct hyperplasia and bile duct epithelial cell necrosis.

HEPATITIS B VIRUS (Moderate concern)

Aetiology: Orthohepadnavirus

Transmission: Infected blood, saliva, semen. Parenteral inoculation or intimate contact required.

Clinical: Infects humans, chimpanzee, gibbon, gorilla, possibly cynomolgus monkey, orangutans and long tailed macaques. Usually no clinical signs other than seroconversion and elevated transaminases. But in advanced cases can cause fibrosis and tumour development.

Diagnosis: Serology or PCR

Pathology: Chronic periportal inflammation with focal hepatocyte necrosis.

HEPATITIS C VIRUS (Low concern)

Aetiology: Flaviviridae

Transmission: Parenteral or sexual contact

Clinical: Only humans and chimpanzees susceptible. Can cause an acute or chronic hepatitis with chronic cases in chimpanzees leading to icterus, lethargy, inappetance and vomiting.

Diagnosis: Serology or PCR

Pathology: Chronic active hepatitis, cirrhosis or hepatocellular carcinoma

STLV-I (Moderate concern in species affected)

Aetiology: Retroviridae subfamily oncovirinae: Type C

Transmission: Parenteral or sexual contact

Clinical: Old world monkeys and apes including baboons, African green monkeys, Patas monkeys, various macaques, and chimps, bonobos and gorilla. Although most infected animals remain latently infected and asymptomatic for life, STLV-I has been associated with lymphoma/leukemia in baboons, African green monkeys, and macaques. Causes Non-Hodgkin's lymphomas.

Diagnosis: Viral isolation, PCR.

Pathology: STLV-I typically infects CD4+ T-cells in macaques and CD8+ T-cells in African monkeys, but some infected T-cell lines express neither marker. STLV-I appears to be nonpathogenic in Asian primates.

NONHUMAN PRIMATE LENTIVIRUSES:

SIV_{mac}	*Macaca mulatta* (Rhesus)
SIV_{smm}	*Cercocebus torquatus atys* (Sooty mangabey)
SIV_{mne}	*Macaca nemestrina* (Pigtailed macaque)
$SIV_{agm/gri}$, $SIV_{agm/tan}$, $SIV_{agm/ver}$	*Cercopithecus sp.* (African green monkey)
SIV_{mnd}	*Papio sphinx* (Mandrill)
SIV_{stm}	*Macaca arctoides* (Stump-tailed macaque)
SIV_{cyn}	*Macaca fascicularis* (Cynomolgus monkey)
SIV_{cpz}	*Pan troglodytes* (Chimpanzee)
SIV_{WCM}	*Cercocebus torquatus lunulatus* (White-crowned mangabey)
SIV_{SYK}	*Cercopithecus mitis* (Sykes monkey)
SIV_{HU}	*Cercocebus Macaca Homo*

SIMIAN IMMUNODEFICIENCY VIRUSES (SIV) (High concern)

Aetiology: Retroviridae subfamily oncovirinae: Type C

Transmission: Direct contact (bite, sexual) and through maternal milk to offspring

Clinical: Documented in African green monkeys, vervets, grivets, tantalus monkeys, Sykes monkeys, mandrills and anubis, sooty, red capped, and white crowned mangabeys, and chimpanzee. Weight loss, diarrhoea, and anaemia/thrombocytopenia are common clinical findings. **Zoonotic**

Diagnosis: RT-PCR, serology, electron microscopy

Pathology: Infected animals lose lymphoid tissues which become depleted and opportunistic infections occur. The most common infections are: CMV, *Candida, M. avium/intracellularae, Cryptosporidium, Pneumocystis, Trichomonas, Plasmodium*, adenovirus, rhEBV, and SV40. Lesions thought to be directly caused by SIV include lymphoid hyperplasia, lymphoid depletion, retroviral pneumonia, retroviral

encephalitis, giant cell disease, aseptic thrombosis, and glomerulosclerosis. Many lesions are dependent on the secondary infections.

MARBURG (High concern)

Aetiology: Filoviridae, Mononegavirales

Transmission: Aerosols and contact

Clinical: In monkeys clinical signs develop within 2-6 days and include fever, anorexia, weight loss, petechial rash, leucopenia, thrombocytopenia, elevated aminotransferase, urea and creatinine levels. **Zoonotic.**

Diagnosis: Virus isolation, IHC, TEM, serology

Pathology: Feulgen stain positive inclusion bodies in hepatocytes. Haemorrhage in lung, liver, spleen and focal necrosis in all organs.

EBOLA (High concern)

Aetiology: Filoviridae, Mononegavirales

Transmission: Aerosols, contact and eating infected meat

Clinical: In chimpanzees, gorillas and monkeys clinical signs develop within 3-9 days and include fever, anorexia, weight loss, nasal discharge, hemorrhagic rash, diarrhoea, thrombocytopenia, elevated LDH. **Zoonotic.**

Diagnosis: Virus isolation, PCR, TEM, serology

Pathology: Amphophilic cytoplasm inclusion bodies, fibrin deposition in splenic cords, lymphoid depletion in splenic white pulp, interstitial pneumonia and bronchiolar/alveolar necrosis, adrenal cortical necrosis, splenomegaly and petechial haemorrhage in many organs.

BACTERIAL

TUBERCULOSIS (High concern). Refer to section 4.3.

Aetiology: *Mycobacterium tuberculosis, M. bovis*, *M. avium-intracellulare*

Transmission: Primarily through aerosols, occasionally nosocomial and rarely peroral

Clinical: Zoonotic and can infect all primates with chimpanzees and members of the Cercopithedidae family often, and rarely Prosimiae. In most cases there are no symptoms other than positive tuberculin skin reactions. In severely affected animals clinical signs include dry, soft chronic cough, lymphadenopathy, wasting, diarrhoea, cutaneous ulcerations, hepato and splenomegaly.

Diagnosis: Difficult to confirm and no test is 100%. Screening tests include intradermal testing with mammalian and avian tuberculin injections, blood rapid tests, and x-rays. Culture from tracheal or gastric lavages and Multiantigen print immunoassay (MAPIA).

Treatment: None. Euthanasia recommended due to zoonotic and multi-drug resistance concerns.

Pathology: Focal granulomatous or miliary lesions in any organ are suggestive of tuberculosis, but lesions are most regularly are found in the lung, liver , lymph nodes
and spleen.

LEPROSY (Moderate concern)

Aetiology: *Mycobacterium leprae*

Transmission: Respiratory, and possibly through skin.

Clinical: Nodular thickening of skin and peripheral nerves. Paralytic deformity of hands and feet. Natural infections in chimpanzee and sooty mangabey (*Cercocebus torquatus atys*).

Diagnosis: This bacterium does not grow *in vitro*. Serology and special stains on tissues.

Treatment: None. Euthanasia recommended.

Pathology: Leprosy is a pathologically complex disease that has a spectrum of lesions that depend on the degree of cell mediated immunity the host is able to mount against *M. leprae*. Natural infections in nonhuman primates have taken the lepromatous form, indicating no CMI. Lesions occur predominantly in the skin and peripheral nerves, particularly in cooler areas (ears, tail, scrotum). Histiocytic infiltrate with variable numbers of lymphocytes and plasma cells in skin and nerves. Acid-fast bacilli demonstrable with Fite-Faraco acid fast stain. Nerve lesions are pathognomonic.

LEPTOSPIROSIS (Moderate concern)

Aetiology: *Leptospira interrogans* subspecies

Transmission: Shed in urine and can survive in soil or alkaline water. Infection occurs via mucous membranes or cutaneous lesions.

Clinical: Most are asymptomatic with infection noted in African green monkeys, orangutans and bush babies based on presence of antibodies. Clinical signs observed range from oedema, vomitus, melaena, jaundice, weakness, lethargy and fever.

Diagnosis: Dark field microscopy, silver impregnation, electron microscopy and serology.

Treatment: If active infection, tetracycline or doxycycline.

Pathology: Usually none in African primates. Icterus, haemorrhage (skin, lymph nodes, heart), liver necrosis, fatty degeneration and necrosis of renal tubules

SHIGELLOSIS (High concern)

Aetiology: *Shigella flexneri, S. sonnei,* others are less common.

Transmission: faecal-oral.

Clinical: Variable. Asymptomatic carriers are common. May have soft stool, fluid diarrhoea, or more commonly, the bloody mucoid diarrhoea of classical dysentery. Monkeys with colitis due to *Shigella* will rapidly dehydrate and die unless treated promptly and vigorously. Shigella affects only primates. Clinical disease is often precipitated by stress.

Diagnosis: Culture

Treatment: Supportive care and antibiotics based on culture and sensitivity testing.

Pathology: The lesions of shigellosis are limited to the colon, may be focal or diffuse, and are characterized by oedema, haemorrhage, erosion & ulceration, and pseudomembrane formation. Microscopically the lesion is purulent, necrotizing colitis, often with crypt abscesses. *Shigella* occasionally causes periodontitis in monkeys.

SALMONELLA (High concern if clinical)

Aetiology: *Salmonella enteritidis, S. typhimurium* (>1800 *Salmonella* spp.)

Transmission: faecal-oral, rodent faeces most common source.

Clinical: All non-human primates, but especially Old World species. Can carry asymptomatically. Sporadic or epizootic. Watery to bloody, mucoid diarrhoea. Occasionally vomiting, abortion and osteomyelitis. May become moribund and die.

Diagnosis: Culture, enzyme immunoassays, immunofluorescence.

Treatment: Supportive care usually results in recovery. If antibiotics are prescribed they should be based on culture and sensitivity testing.

Pathology: Necrotizing, suppurative enterocolitis. May become septicaemic resulting in pyogranulomas in liver and other organs. Resembles shigellosis, but *Shigella* does not become septicaemic and does not affect the small intestine.

CAMPYLOBACTERIOSIS (Moderate concern if clinical)

Aetiology: *Campylobacter. jejuni, C. coli*

Transmission: Oral via contaminated food or water

Clinical: Asymptomatic carriers are common. Diseased monkeys have fluid, sometimes bloody diarrhoea and dehydration. *Campylobacter* has been associated with abortions in primates.

Diagnosis: Isolation requires special media and atmosphere, PCR or serology.

Treatment: Usually supportive care. If antibiotics are required they should be selected based on culture and sensitivity testing as multi-drug resistance is common. Erythromycin, Tetracycline and Quinolones commonly used.

Pathology: Small intestine and colon reddened, roughened, oedematous. Histology in colon can be similar to shigellosis, but is usually much less severe and can also affect small intestine. Colonic mucosa sometimes hyperplastic. Can demonstrate spiral bacteria with silver stains.

HELICOBACTERIOSIS (Low concern)

Aetiology: *Helicobacter pylori*

Transmission: Oral (oral-oral or faecal-oral) and possibly via waterborne infections

Clinical: Asymptomatic usually. Clinical signs range from intermittent vomiting and diarrhoea.

Diagnosis: Can see organism with HE, but Giemsa or silver stains will more readily demonstrate slightly curved, rod-shaped, gull-wing, or loosely-coiled organisms, 1-4 μm long, associated with gastric epithelium in antral mucosa. Best to culture biopsy rather than swab, are urease positive. Can use rapid urease test rather than culture.

Treatment: Antibiotics and anti-ulcer medications.

Pathology: Seldom grossly apparent, but sometimes focal reddening or erosions of gastric mucosa. Mononuclear inflammatory cell infiltrate in lamina propria of stomach, superficial erosions, epithelial hyperplasia.

STREPTOCOCCUS PNEUMONIAE (DIPLOCOCCUS) (Moderate concern)

Aetiology: *Streptococcus (Diplococcus) pneumoniae.*

Transmission: Aerosols

Clinical: Tends to occur in small focal outbreaks. Often found dead, but may have signs of pneumonia, meningitis, arthritis, depression, dehydration, paralysis, tremors or seizures.

Diagnosis: Culture, PCR

Treatment: Antibiotics (penicillin, cephalosporin and macrolide resistance is common) based on culture and sensitivity testing.

Pathology: Fibrinopurulent serositis affecting meninges, pleura, peritoneum, and/or joints. Often severe fibrinopurulent pneumonia. Sometimes only septicaemia, especially if splenectomised. Numerous thrombi and infarcts - can result in permanent CNS damage if survive. Diplococci easy to see on gram stained smear of exudates.

YERSINIOSIS (High concern if clinical)

Aetiology: *Yersinia pseudotuberculosis, Y. enterocolitica.*

Transmission: Wild birds and rodents are reservoir hosts. Transmission by ingestion of feed/water contaminated by faeces of infected animals.

Clinical: Disease of all non-human primates. Affected primates are often found dead but sometimes show diarrhoea, depression, and dehydration. *Yersinia* is occasionally associated with abortions and stillbirths.

Diagnosis: Culture, PCR, serology

Treatment: Supportive care. If antibiotics are required they should be selected based on culture and sensitivity testing as multi-drug resistance is common.

Pathology: The infection begins as a focal necrotizing enteritis and mesenteric lymphadenitis, which rapidly becomes septicaemic resulting in necropurulent hepatitis, splenitis, and myelitis. Large colonies of gram negative bacteria in necrotic centres are nearly diagnostic.

LISTERIOSIS (Moderate concern)

Aetiology: *Listeria monocytogenes*

Transmission: *Listeria* is widespread in the environment. Oral from contaminated food and transplacental transmission.

Clinical: Disease occurs in stillborn and neonatal infants. Abortion, intrauterine death, neonatal sepsis, meningoencephalitis in infants. Mother usually clinically normal.

Diagnosis: Culture, serology

Treatment: Antibiotics (Ampicillin, penicillin, tetracycline, erythromycin) based on culture and sensitivity.

Pathology: Purulent placentitis (haematogenous pattern), purulent meningo-encephalitis, intrauterine pneumonia, focal necrosis in liver and other organs, gram-positive rods in tissues.

BORDETELLOSIS (Low concern)

Aetiology: *Bordetella bronchiseptica*

Transmission: Respiratory

Clinical: Asymptomatic carriers. Mucopurulent nasal discharge, dyspnea, and sometimes peracute death.

Diagnosis: Culture, serology

Treatment: Antibiotics (Polymyxin B, Tetracycline, Gentamycin, Kanamycin) based on culture and sensitivity.

Pathology: Fibrinopurulent hemorrhagic bronchopneumonia. Fibroplasia around bronchioles.

TETANUS (Moderate concern)

Aetiology: *Clostridium tetani*.

Transmission: *C. tetani* is a soil organism and an obligate anaerobe that contaminates skin wounds or bites

Clinical: Begins in upper limbs, then lower. Deliberate stiff gait, trismus, extensor rigidity, opisthotonos and death. Usually fatal in l-10 days due to respiratory paralysis and exhaustion. Tetanus is a non-immunizing disease - multiple episodes are possible. Antibody is not usually detectable in affected animals.

Diagnosis: Usually based on clinical signs as culture is extremely difficult

Treatment: Hyperimmuneserum, Penicillin, Tetracyclines

Pathology: None. Must be diagnosed clinically.

STAPHYLOCOCCUS (Moderate concern)

Aetiology: *Staphylococcus aureus*

Transmission: *Staphylococcus* is commonly carried asymptomatically in the nose and throat but occasionally infects breaks in the skin and invades the bloodstream.

Clinical: Pustular dermatitis in young animals. Breaks in skin become infected resulting in cellulitis, abscesses, and lymphadenitis. Bacteraemia often develops, leading to visceral abscesses, endocarditis, and septic shock. Vegetative valvulitis may cause septic emboli and infarcts in various organs. Indwelling catheters are a common source of infection. The source of infection is usually clinically obvious.

Diagnosis: Culture

Treatment: Antibiotics based on culture and sensitivity testing.

Pathology: Cellulitis, abscesses filled with thick creamy pus, fibrinous pericarditis, vegetative valvulitis, thrombosis and infarction. Histological lesions consist of

fibrinopurulent exudate with masses of gram-positive cocci. Monkeys sometimes develop secondary immune complex glomerulonephritis.

KLEBSIELLA (Moderate concern)

Aetiology: *Klebsiella pneumoniae*

Transmission: Respiratory. Carried in nose and throat. Often seen in chimpanzees

Clinical: Nasal discharge, air sacculitis, bronchopneumonia, meningitis, arthritis, cystitis

Diagnosis: Culture

Treatment: Supportive care. Bacterium is resistant to most antibiotics and therefore if antibiotics are prescribed they should be selected based on culture and sensitivity testing.

Pathology: Fibrinopurulent pneumonia and serositis, septicaemia. Abundant gram-negative bacteria with prominent capsules in exudate. Exudate sometimes has a gelatinous consistency.

ESCHERICHIA COLI (High concern for highly pathogenic strains)

Aetiology: *E. coli* (serotypes 0119:B14, 055:B5, 026:B6)

Transmission: Faecal-oral

Clinical: All non-human primates. Occurs as sporadic cases but often a peracute disease. Pneumonia, meningitis, diarrhoea, lethargy and dehydration.

Diagnosis: Culture

Treatment: Aggressive antibiotic therapy and prophylactic antibiotics to exposed individuals. Vaccination is ineffective.

Pathology: Fibrinopurulent pneumonia and serositis, pyelonephritis, hemorrhagic gastroenteritis.

MELOIDOSIS (Moderate concern)

Aetiology: *Pseudomonas (*aka *Burkholderia) pseudomallei* (A saprophyte with worldwide distribution)

Transmission: Most cases are due to contamination from bites or other wounds. Occasionally inhalation and ingestion have been reported with a prolonged incubation period (3 months to 10 years!)

Clinical: All non-human primates. Infection can involve any organ system and therefore clinical signs are based on the organs infected. Most commonly infected are lung, liver and bone.

Diagnosis: Culture, PCR and serology

Treatment: Usually unsuccessful due to multi-drug resistance and ability to quickly acquire resistance during the course of treatment.

Pathology: Yellowish coloration of the subcutaneous tissues, white, milky subcutaneous abscesses. Acute necrotizing-glaucomatous inflammation containing giant macrophages with phagocytes leukocytes and intracellular bacteria.

FUNGAL DISEASES

DERMATOMYCOSIS (Moderate concern)

Aetiology: *Trichophyton rubrum* and *Microsporum canis*

Transmission: Contact with an animal or fomite

Clinical: Circular lesions on the skin with alopecia.

Diagnosis: Culture and direct microscopic examination of hairs with characteristic hyphae or arthrospores

Treatment: Systemic anti-fungals (ketoconazole, itrarconazole, lefuronon, griseofulvin, terbinafine)

Pathology: Folliculitis, alopecia and clusters of arthrospores in keratinized tissues.

CANDIDIASIS (Moderate concern)

Aetiology: *Candida albicans*

Transmission: Ubiquitous organism. Opportunistic infection of immuno-compromised animals.

Clinical: Occurs in debilitated animals or animals on long-term antibiotics. Dysphagia, white pseudomembrane on oral mucous membranes.

Diagnosis: Culture**.** Organism can be seen on HE, but best studied with PAS or GMS stains

Treatment: Nystatin, ketoconazole, itraconazole, use with probiotics.

Pathology: White pseudomembrane on oral and esophageal mucous membranes. Underlying tissue may be ulcerated. Septate pseudohyphae and oval budding blastospores in superficial epithelium. Rarely invade past basement membrane.

PNEUMOCYSTIS (Moderate concern)

Aetiology: *Pneumocystis carinii* (Unculturable fungus between ascomycetes and basidiomycetes)

Transmission: Aerosol

Clinical: Often asymptomatic. Horizontally acquired or reactivated latent infections in immunodeficient animals. Fever, dyspnea, cough, anorexia and weight loss.

Diagnosis: Histopathology (Methenamine-silver staining), IHC, PCR

Treatment: Trimethoprim-sulfa

Pathology: Lesions usually restricted to lung, but generalized infections have been described in severely immunodeficient humans. Foamy eosinophilic intra-alveolar exudate mixed with alveolar macrophages, interstitial lymphocytic infiltration, hypertrophy of alveolar lining cells. Organisms in exudate and along alveolar walls. Cysts demonstrable with GMS stain.

HISTOPLASMOSIS (Low concern)

Aetiology: *Histoplasma capsulatum* var *capsulatum*, *Histoplasma capsulatum* var *duboisii* (African histoplasmosis)

Transmission: Inhalation of spores of *H capsulatum* from soil rich in bird or bat excreta. *H. duboisii* may be spread by dermal contact and may have a long incubation period.

Clinical: *H. capsulatum*: Infection is usually latent. Clinical disease can occur with heavy exposure, resulting in influenza-like disease, with pre-existing lung lesions, or disseminated disease can result from immunodeficiency. Rare in monkeys.
H. duboisii. Reported only from Africa in humans and baboons. Affects skin, lymph node, and bone.

Diagnosis: Sometimes with fine-needle aspirates. Tissue biopsies with PAS, methenamine silver or Gridley's fungal stains.

Treatment: Itraconazole, ketoconazole or in difficult cases amphotericin B.

Pathology: Granulomatous inflammation in affected organs, histiocytes packed with yeast form of organism. *H. capsulatum* 2-4µ, *H. duboisii* 7-15µ.

CRYPTOCOCCOSIS (Low concern)

Aetiology: *Cryptococcus neoformans*

Transmission: Inhalation

Clinical: CNS and ocular abnormalities usually.

Diagnosis: Cytology with gram stain (retains crystal violet and capsule stains lightly red), culture and serology.

Treatment: Fluconazole, itraconazole, amphotericin B.

Pathology: Gelatinous nodules or cystic areas, especially on meninges, grossly. Sparse granulomatous inflammation surrounding abundant yeasts. Organism is 5-10μ, has single buds, and is surrounded by a thick mucin positive capsule.

PARASITES (see also sections 3.10, 3.11)

GIARDIA (Moderate concern)

Aetiology: *Giardia intestinalis*

Transmission: Faecal-oral, contaminated water

Clinical: Diarrhoea (small intestinal), vomiting

Diagnosis: Characteristic trophozoites or cysts in faeces, fluorescent antibody, faecal antigen

Treatment: Isolation and treatment of infected animals with metronidazole

Pathology: Presence of organisms on epithelial surface of small intestine. Organisms most common in middle of jejunum. Mucosa may be normal or have nonspecific villus atrophy and inflammation of lamina propria.

AMEBIC DYSENTERY (High concern)

Aetiology: *Entamoeba histolytica*

Transmission: Faecal-oral

Clinical: Lethargy, weakness, dehydration, anorexia, vomiting, severe diarrhoea with blood and mucus present

Diagnosis: Faecal smears, IF, RIA's

Treatment: Imidazole derivatives (Metroniadazole, etc.)

Pathology: Cecocolic necrotic areas with occasional sunken centres, chronic colitis and in chimpanzees, pulmonary abscesses. Colobinae have ulcerative gastritis and hepatic abscesses

AMEBIC MENINGOENCEPHALITIS (Low concern)

Aetiology: *Balamutharis mandrillaris*

Transmission: Unknown

Clinical: Cutaneous granulomas, limb paresis or paralysis, depression and weakness

Diagnosis: Isolation of the organism from brain/skin tissue. Methenamine silver or PAS staining.

Treatment: Clarithromycin, fluconazole, sulfadiazine, flurocytosine.

Pathology: Random multimodal encephalomalacia and cerebral haemorrhage. Nodular necrosis of the liver, kidney, lung and pancreas. Presence of trophozoites and cysts in the affected neuropil.

MALARIA (Moderate concern)

Aetiology: *Plasmodium sp.*

Transmission: Mosquito bites

Clinical: Asymptomatic. Weakness due to anaemia

Diagnosis: Blood smear identification of the organism in erythrocytes

Treatment: Not required in great apes due to mild disease

Pathology: Grossly, the lungs, liver, and spleen are gray and the blood thin. Histologically, tissue macrophages are filled with malarial pigment, and there are hemosiderosis and parasitized RBC's. Intravascular clotting with thrombi and parasitized RBC's is common. Often there is pulmonary and cerebral edema.

BALANTIDIASIS (High concern – but species dependant)

Aetiology: *Balantidium coli*

Transmission: Faecal-oral

Clinical: Weight loss, anorexia, weakness, watery diarrhoea, tenesmus, rectal prolapse

Diagnosis: Fresh faecal sample (wet prep and concentration)

Treatment: Imidazole derivatives (Metronidazole), tetracycline or doxycycline

Pathology: Organisms are often found in the lumen of the colon of normal animals and in ulcerative lesions, mucosa, capillaries, lymphatics, and mesenteric lymph nodes of animals with colitis. Usually associated with some other pathogen. May be a primary pathogen in great apes.

STRONGYLOIDOSIS (High concern)

Aetiology: *Strongyloides stercoralis, S. fulleborni, S. cebus*

Transmission: Faecal-oral (transmucosal) usually via soil, percutaneous and transplacental

Clinical: Diarrhoea with or without blood and mucus, urticaria, anorexia, depression, listlessness, vomiting, emaciation, constipation, dyspnea, cough, death

Diagnosis: Faecal concentration and culture with typical larvae (*S. stercoralis*) or eggs (*S. fulleborni, S. cebus*) in stool

Treatment: Benzimidazole, ivermectin, pyrantel pamoate or levamizole

Pathology: Multifocal or diffuse pulmonary haemorrhage, catarrhal to hemorrhagic-necrotic enterocolitis. In hyper infections there is sub acute eosinophilic interstitial pneumonia, eosinophilic vasculitis and perivasculitis.

NODULAR WORM (High concern)

Aetiology: *Oesophagostomum sp.*

Transmission: Faecal-oral

Clinical: Low burdens are usually well tolerated. Unthriftiness and debilitation with weight loss and diarrhoea and death in heavy infestations.

Diagnosis: Faecal concentration with identification of eggs. Note: *Oesophagostomum sp.* are indistinguishable from certain strongyles (*Ternidens)* and hookworm eggs. PCR-RFLP

Treatment: Benzimidazole, ivermectin, pyrantel pamoate or levamizole

Pathology: 2-5mm diameter nodules in the sub mucosal layer of the large intestine. On cut surface the nodules are filled by a brown, creamy material and connected to the intestinal lumen by a small ulcer)

HOOKWORM (High Concern)

Aetiology: *Ancylostoma duodenal, Necator americanus*

Transmission: Faecal-oral for both parasites plus cutaneous penetration for *Necator*

Clinical: Anaemia, weakness, distended abdomen, dyspnea on exertion and general debilitation.

Diagnosis: Identification of eggs in faeces which should be cultured for definitive diagnosis.

Treatment: Benzimidazole, ivermectin, pyrantel pamoate or levamizole

Pathology: Adults found attached to the small intestine at necropsy with intestinal hyperaemia, ulceration and oedema. The liver and other organs may be pale. *Necator* larvae can be found in the lungs and heart.

PINWORM (Moderate concern)

Aetiology: *Enterobius sp. (E. vermicularis* is zoonotic), *Colobenterobius* sp., *Prostmayria* sp., *Lemuricola* sp.

Transmission: Faecal-oral

Clinical: Perianal pruritus that can lead to self mutilation, restlessness and increased aggression

Diagnosis: Observing the adult worms emerging from the anus or from a cellophane tape impression. Egg identification on faecal concentration

Treatment: Benzimidazole, ivermectin, pyrantel pamoate or levamizole

Pathology: Cecal/colonic/rectal mucosal hyperaemia. Oedematous thickening or fibrosis of the large intestinal wall.

WHIPWORM (Moderate concern)

Aetiology: *Trichuris trichura*

Transmission: Faecal-oral

Clinical: Anorexia and diarrhoea in severe infections

Diagnosis: Identification of eggs on faecal sample (concentration technique)

Treatment: Benzimidazole, ivermectin, pyrantel pamoate or levamizole

Pathology: Adults found with anterior end embedded in mucosa of cecum and proximal colon. Little host reaction. Heavy infestations rarely associated with intussusception.

HYDATID DISEASE (Low concern)

Aetiology: *Echinococcus sp.*

Transmission: Faecal-oral (canid/feline faeces)

Clinical: Abdominal distension, exophthalmia, localized subcutaneous swellings. Sudden death in pulmonary echinococcosis due to anaphylactic shock.

Diagnosis: Ultrasonography; aspiration of cyst and identification of scolices in aspirate

Treatment: Mebendazole, Albendazole

Pathology: Cyst formation in abdominal, thoracic or pelvis organs that contain viable scolices that are surrounded by a thick laminated membrane.

LUNG MITE (Moderate concern)

Aetiology: *Pneumonyssus sp.*

Transmission: Oro-nasal from mother to offspring

Clinical: Paroxysms of coughing or sneezing. Death from massive infestation in chimpanzees.

Diagnosis: Identification of the egg or larvae in bronchial wash, faeces or at necropsy

Treatment: Ivermectin

Pathology: 1-2mm diameter yellowish-white nodules due to focal chronic endo and peribronchiolitis. Adult lung mites in bronchoiectatic caverns, per bronchiolar and per vascular macrophages filled with yellow-brown pigment.

SCABIES (Moderate concern)

Aetiology: *Sarcoptes scabiei*

Transmission: Direct skin contact

Clinical: Intense pruritus, anorexia, weakness, weight loss, emaciation and localized to generalized alopecia

Diagnosis: Identification of the mite on deep skin scrapings

Treatment: Ivermectin

Pathology: Characteristic appearance of mites and eggs within the stratum corneum.

SCHISTOSOMIASIS (Moderate concern)

Aetiology: *Schistosoma mansoni, S. hematobium*

Transmission: Direct skin contact

Clinical: Anaemia, ascites, hepatomegaly, emaciation, anorexia and death.

Diagnosis: Identification of the eggs in faecal samples. Serology.

Treatment: Praziquantel

Pathology: Periportal hepatic fibrosis, hepatomegaly, colonic mucosal erosions.

PERIODONTAL DISEASE (Low concern)

Aetiology: Unknown, various bacteria including Shigella sp. occasionally, NOMA associated with SAIDS.

Clinical: Usually none. Animals often debilitated or immunosuppressed due to other causes. Associated with dental calculus.

Pathology: Lesions vary from slight reddening of gums to necrotizing ulcerative gingivitis. Gingival bleeding common. Interproximal craters with alveolar bone destruction in severe cases. Spirochetes can be demonstrated in gingival connective tissue by silver staining. *Shigella flexneri*, serotype 4 is commonly present. Gangrenous necrosis of bone and overlying soft tissues in NOMA (Cancrum oris) - associated with type D retrovirus infection has been reported in macaques.

FIBROUS GINGIVAL HYPERPLASIA (Low concern)

Aetiology: Unknown. May be sequelae to chronic gingivitis. Possibly familial in macaques. Phenytoin may also produce gingival enlargement.

Clinical: Usually none.

Pathology: Mild to marked firm enlargement of the marginal and alveolar gingiva, including the
interdental papillae. The hyperplastic tissue is normal in colour and may completely cover the teeth. Microscopically, the tissue is dense collagen with little inflammation.

ACUTE GASTRIC DILATATION (BLOAT) (High Concern)

Aetiology: Thought to be multifactorial including food restriction, overeating, anesthesia. Usually occurs in caged monkeys. *Clostridium perfringens Type A* can be isolated in large numbers and may be responsible for gas production.

Clinical: Old world monkeys. Usually found dead with enlarged and firm abdomens.

Pathology: Stomach markedly distended with gas and brown watery fluid, intestine congested.
Subcutaneous emphysema occurs if the stomach ruptures. Affected monkeys die of respiratory
embarrassment, impaired venous return, and shock.

DIVERTICULOSIS (Moderate concern)

Etiology: Unknown. Possibly congenital or due to spastic contraction of the colon

Clinical: Usually none. Abdominal pain.

Pathology: Saccular protrusions along taenia coli, muscular hypertrophy. May become impacted and inflamed.

CARDIOMYOPATHY (High Concern)

Aetiology: Unknown.

Clinical: Gorillas and chimpanzees: Common from middle age onward, associated with obesity. May cause no clinical signs or sudden death, especially during anesthesia.

Pathology: Cardiomegaly, myocardial hypertrophy, fibrosis, atrial thrombosis, saddle thrombus in aorta, ascites, pulmonary oedema. Myocardial fibrosis and hypertrophy in apes.

IRON STORAGE DISEASE (Low concern)

Aetiology Excessive iron accumulation leading to pathological conditions

Clinical: Associated with concurrent diseases, aging, or wasting. Seen in gorillas. Liver most affected tissue. Also gut, spleen, kidney, heart and pancreas.

Pathology: Copper or bronze coloured swollen liver. Sometimes some scarring and cirrhotic appearance. Yellow/gold pigment in cytoplasm of hepatocytes, Kupffer cells, renal tubular epithelium splenic reticular cells, myocardiocytes, pancreatic acinar cells. In hemochromatosis, liver has bridging fibrosis, biliary hyperplasia, hepatocellular cytomegalic and karyomegalic change and necrosis.

ENDOMETRIOSIS (Moderate concern)

Aetiology: Implantation of normal endometrial tissue in ectopic locations. Several theories as to how this occurs but none conclusive.

Clinical: Depends on site of implantation. Abdominal swelling, constipation, uraemia.

Pathology: Multiple cysts which contain "chocolate brown" fluid, extensive fibrosis, hemosiderosis, and adhesions. The lower abdominal and pelvic organs are often adhered into a single mass. Can cause obstruction of intestine and ureters. Histologically, endometrial epithelium and stroma must be present to differentiate endometriosis from endometrial carcinoma. Dense fibrosis and hemosiderosis are usually present.

ARTHRITIS (Moderate concern)

Aetiology: Associated with calcium pyrophosphate crystal deposition in joint.

Clinical: Enlarged joints, muscle contracture and wasting.

Pathology: Can affect any joint. Interphalangeal joints and knees most obviously affected. Reducedsynovial fluid, cartilage erosion and ulceration, synovial hyperplasia, neutrophilic infiltrates. Calcium pyrophosphate crystals can be observed in articular surfaces by SEM in many cases.

TRAUMA DUE TO FIGHTING (Moderate concern)

Etiology: Fight injuries in group housed animals, particularly in breeding season or when new animals are added to group.

Clinical: Lacerations, bruises, abrasions, punctures, etc. Injuries to underlying soft tissue are often more extensive than is apparent from the appearance of the skin lesions. Gangrene of distal extremities can develop due to extensive crushing and bacterial contamination.

Pathology: Extensive muscle necrosis, gangrene, myoglobinuric nephrosis, hyperkalemia.

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4.2 RESPIRATORY INFECTIONS IN PRIMATES

Chris Colin

Tuberculosis is not included here, as addressed elsewhere in the manual, but tuberculosis is a major cause of respiratory disease.

- Brief overview of case studies of respiratory outbreaks in wild apes
  a. Principles of studies

An increasing number of studies have now shown a link between respiratory disease outbreaks in groups of wild habituated apes and human pathogens. These studies provide data on the health status of individual apes, faecal sample analysis, and necropsy and tissue analyses. Isolated pathogens were then identified phylogenetically. The results revealed that human pathogens were clearly implicated in both morbidity and mortality of great apes, thus highlighting the high probability that the infected apes were contaminated by researchers studying them, tourists or local people living close by.

b. Human Pathogens linked to RD outbreaks in wild apes

The human pathogens found were:

- Human Respiratory Syncitial Virus
- Human Metapneumovirus (HMPV)
- *Streptoccus pneumoniae*

} Human paramyxoviruses. **Humans are the only known reservoir for both viruses**

Example of results from different groups of wild habituated chimpanzees in the Taï Forest (Côte d'Ivoire) who have experienced respiratory disease outbreaks

	May 1999	March 2004	February 2006	
Group	***North***	***South***	***South***	***East***
Pathogens identified	**HRSV**, *S.pneumoniae* **strain 2308**	**HMPV**, *S.pneumoniae* **strain 2309**, *P.multocida*	**HRSV**, *S.Pneumoniae* **strain 2309**	**HSRV**, *S.pneumoniae* **strain 2308**
Morbidity	**100%**	**100%**	**92%**	ND
Mortality	6/32 (**19%**)	8/44 (**18%**)	1/34 (**3%**)	2/?
Adult & adolescent (≥15 ; 10 – 15)	5/15	0/22	0/19	2
Juvenile (5 – 10)	1/7	3/10	0/5	0
Infant (0 to 5)	0/10	5/12	1/10	0

Köndgen et al., Pandemic human viruses cause decline of endangered great apes, Current Biology (2008), doi:10.1016/j.cub.2008.01.012

Presentation of these 3 pathogens

- **The Human Respiratory Syncitial Virus (RSV) is the most important cause of viral respiratory tract illness in infants and children worldwide**, according to the Center

for Diseases Control and Prevention (CDC). RSV's tropism is directed at epithelium in the respiratory tract and can cause rhinitis, otitis media, pneumonia or bronchiolitis. **Humans are the only known reservoir for RSV**. Spread of the virus from contaminated nasal secretions occurs via respiratory droplets, and close contact with an infected individual or with a contaminated surface. **The virus can persist for several hours on objects and surfaces.**

In developing countries, there are few studies on the incidence of RSV in respiratory diseases but **existing data show that RSV accounts for a high proportion of respiratory infections in children**. Researchers estimate that RSV around the world is responsible for 64 million cases of respiratory disease and 160 000 deaths every year.

Studies showed that **naturally acquired immunity to RSV is neither complete nor durable** and recurrent infections occur frequently during the first three years of the life. Adults and older children are usually protected against severe RSV infection which suggests that resistance develops after primary infection.

- **Human Metapneumovirus (HMPV)** was discovered and described only recently in 2001 by researchers in the Netherlands. Since this first study, HMPV has been identified in countries all over the world except Antarctica. There are two major groups (A and B) and four subgroups for this virus.

  The pathophysiology of HMPV infection is thought to be closely related to RSV. Like RSV, HMPV has a tropism for the respiratory epithelium. The patient may be asymptomatic, or symptoms may range from mild upper respiratory tract symptoms to severe bronchiolitis and pneumonia. Some studies had shown that HMPV is the second most commonly identified cause of lower respiratory illness in children, after RSV. It also causes disease in all age groups. **Usually HMPV infection occurs early in childhood and the seroposivity rate approaches 100% by 10 years old**. Re infection is however possible as HMPV infections had been seen in adults patients. Elderly and immuno-compromised patients are also at greater risk of contracting HMPV.

  A really interesting point is that multiple animal models have been used to study the pathophysiology of HMPV and **chimpanzees have been the only animal to demonstrate symptomatology consistent with human disease.**

  There is currently no vaccine for HMPV.

- ***Streptococcus pneumoniae*** is a Gram-positive aerobic encapsulated diplococcus. The differences in the composition of the capsule have permitted the identification of 90 serotypes, but only about 11 of the most common serotypes seem to be involved in 75% of the pneumoccocal diseases.

  Pneumococci commonly colonize the human respiratory tract (nasopharynx region) and are transmitted exactly in the same way as RSV and HMPV are, by direct contact with respiratory secretions. Young children are common healthy carriers.

  When exposed to Pneumococci, an individual might have a transient nasopharyngeal colonization without expressing the disease, but the bacteria can spread to the sinuses or the middle ear or a bacteriema is possible if the bacteria have penetrated the mucosal layer, when the individual is susceptible to the particular serotype and when his/her immune system is depressed by other pathogens (e.g. HMPV or RSV). Pneumococci can **cause upper respiratory tract infections and can also result in pneumonia, meningitis and septicaemia**. In developed countries it affects more the elderly population while it affects the youngest children in developing countries.

Nowadays *Streptococcus pneumoniae* is resistant to many antimicrobials (penicillins, cephalosporins and macrolides) and it is a serious health problem worldwide. Treatment therefore requires nowadays stronger doses of antibiotics taken over a longer period of time.
It is a major cause of morbidity and mortality among children worldwide, especially in developing countries. **Some studies have estimated that more than 10 million children less 5 years old are infected with pneumococcal disease every year**.

There is a polysaccharide vaccine against *Streptococcus pneumoniae* but this does not appear tio be effective in non human primates.

These 3 pathogens spread from one individual to another through direct physical contact with secretions or droplets and are responsible for serious disease outbreaks of concern worldwide.

c. Consequences

- Many long-term field sites of wild great apes have now put in place **strict protocols both for researchers and tourists**: compulsory wear of masks, strict minimal observation distances to be maintained between humans and great apes, no sick human is allowed to go and approach the apes, no human wastes are allowed in the forest, etc. An increasing number of sites are also developing health community programs for local people surrounding wild great ape populations.

- As these human pathogens are responsible for many of the respiratory outbreaks witnessed in groups of habituated great apes, it is clear that the risks as extremely elevated in sanctuaries caring for rescued great apes, where contact between the animals and staff is extremely frequent and direct physical contact is common (e.g. during nursing, feedings, veterinarian care, rehabilitation of young orphans etc.). In addition, there is a significant risk that rescued individuals are infected with or carrier of such pathogens, as the probability of them contracting these pathogens whilst in captivity, often in poor conditions, is often extremely high.

- This means that **strict hygiene and health protocols and implementation of quarantine procedures must be implemented to prevent the risk of spread of pathogens to both humans and great apes in sanctuaries.**

➢ INFECTIONS OF THE UPPER RESPIRATORY TRACT (URT)

a. Definition of URT

The upper respiratory tract is composed of the sinuses, nasal passages, pharynx, and larynx.

b. Pathogens (in humans and apes)

Primary infections of URT are mostly caused by viruses in Humans. These include rhinoviruses, coronaviruses, adenoviruses, paramyxoviruses or coxsackieviruses.

Bacterial primary infections are possible, although they are often secondary, especially in young or immuno-compromised individuals. *Sterptococcus pneumoniae* is one of the numerous bacteria responsible for such infections.

c. Physiopathology

The pathogens are transmitted through physical contact with secretions or droplets produced when an infected individual sneezes or coughs. The pathogens then invade the mucosa of the URT, causing local inflammation and an immune reaction.
The frequent changes of the antigenicity of the viruses involved pose challenges to the immune system and their resistance to destruction (by production of toxins, proteases, etc.) is particularly challenging when treating weak or young individuals.
The **most common symptoms** vary from **nasal discharge** and **congestion**, **cough**, **sneezing**, **sore throat**; **fever is most common in children**. In human children, such infections can be serious when it involves the larynx area which is much smaller than in an adult, and this can dangerously compromise the airflow.
A URI can easily spread and evolve into pneumonia.
We have all experienced in sanctuaries how young primates are extremely sensitive to simple URI and how URI can rapidly result in more serious respiratory infections if gone untreated.

Incubation times before the apparition of symptoms vary from 1 to 14 days depending on the pathogen.

d. Treatment

The treatment is symptomatic when the symptoms are moderate.

- **Analgesics** like acetaminophen can be useful to reduce the pain (do not use Aspirin on young individuals because of the risk of Reye Syndrom – see Rosa Garriga's case study 2008)
- **Antipyretic if fever** and be sure the individual drinks enough (water, fruit juices for example)
- **Decongestants PO or topical** (pseudoephedrine, phenylephrine), but be careful with side effects on young individuals: these include burning of mucosa, sneezing, increased nasal discharge and when given PO, side effects may include restlessness, anxiety, nervousness, weakness and difficulty breathing
- **Corticosteroids in nebulization** to decrease edema by suppressing the local inflammation **but do not use if there is any suspicion of bacterial or fungal infection!**

In more severe cases, and if there is any suspicion of bacterial co-infection:

- **Epinephrine** (nebulization) can help **in cases of severe bronchostriction**
- Antibiotherapy: Penicillin G, **amoxicillin**, **amoxicillin + clavulanic acid**, **cephalosporins of 2nd generation**, **erythromycin**, **azythromycin** are the most used to treat co-infections in URI

e. Prevention

Prevention measures must be put in place to protect the animals and sanctuary staff and visitors must adhere to some basic prevention rules.

- **For the primates**:

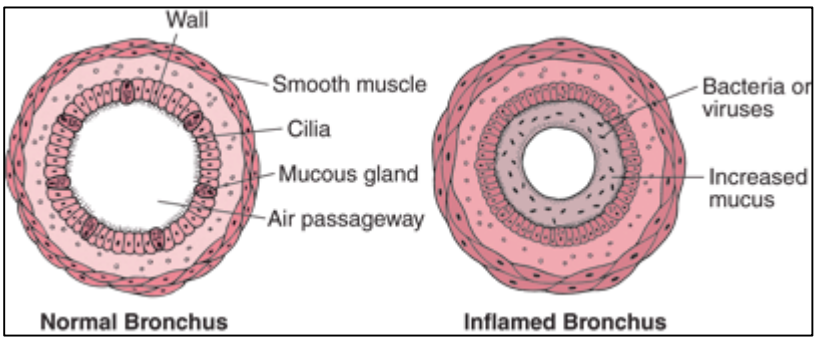
- ✓ **Respect quarantine procedures for all new primates**. Babies and juveniles who were in captivity in poor condition are often sick with respiratory diseases. Avoid contact with other primates until the individual has been treated or ensure that the individual does not exhibit respiratory symptoms during the whole quarantine period (*do not forget that incubations time can be as long as 2 weeks*)
- ✓ Whenever there is a case of URI in a group, try to isolate the sick individuals if possible. If not possible, be really careful to monitor the evolution of the disease, especially in young primates. Do not hesitate to treat, symptomatically first, but also with antibiotics in severe cases or if complications arise.
- ✓ You can supplement primates' food with multi-vitamins and make sure they receive food of really high quality especially if there is a loss of appetite
- ✓ Maintain **good hygiene standards for all the infrastructures** and any equipment used for the primates (e.g. buckets)

- **For the staff and visitors**:
  - ✓ **Any staff member showing signs of URI should not have contact with the primates** or equipment used for the primates.
  - ✓ When there is an outbreak of URI in the animals, staff should wear masks (and gloves if possible) to prevent zoonotic transmission
  - ✓ If you receive visitors in your sanctuary, make sure you have strict rules concerning the distance to respect between visitors and primates. Visitors must not give food to the primates.
  - ✓ **Be really careful with children visiting the sanctuary**. In general, if a child shows any signs of respiratory disease or childhood disease, he/she should not be allowed to enter in the sanctuary.

➢ INFECTIONS OF THE LOWER RESPIRATORY TRACT

a. Bronchitis

Bronchitis is an inflammation of the bronchi usually following an infection of the upper respiratory tract. Acute bronchitis is often caused by a virus which first caused an URI. It is rarely caused by bacteria. It can also be caused by the inhalation of food or vomit or irritating products (such as smoke in humans). The inflamed bronchi produce mucus.



http://www.merck.com/mmhe/sec04/ch041/ch041a.html

- The symptoms generally appear few days after an URI and include a dry cough at the beginning, but it is usually productive after few days, with production of white, then yellow/green mucus. This kind of cough can last for several weeks. There can be a mild fever (<101°F or <38.3°C), chest pain, tiredness, wheezing noises.

  It is really important to differentiate bronchitis from pneumonia, where the symptoms are high fever, shortness of breath, shaking chills, etc.

- There is no treatment when a virus is the cause of bronchitis, it disappears generally by itself. Give plenty of fluids to help thinning of the secretions. In cases of suspicion of bacterial infection, try to make a sputum culture and give antibiotics if necessary.

- Supportive treatment: antipyretic, expectorants.

- Prevention is the same as for URI.

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b. Pneumonia

"Pneumonia is an inflammation of the lung parenchyma characterized by consolidation of the affected part, the alveolar air spaces being filled with exudate, inflammatory cells, and fibrin. Most cases are due to infection by bacteria or viruses, a few to inhalation of chemicals or trauma to the chest wall, and a few to rickettsiae, fungi, and yeasts. Distribution may be lobar, segmental, or lobular; when lobular and associated with bronchitis, it is termed bronchopneumonia."
http://dictionary.webmd.com/terms/pneumonia

When pathogens enter the lungs, they reach the space between alveoli (bacteria and fungi) or invade the pulmonary cells (virus). In case of bacteria or fungi, the immune system of the body responds by sending neutrophils which destroy the pathogens, releasing cytokines and leading to an inflammatory response (then you have appearance of general symptoms such as fever, chills, cough, chest pain, shortness of breath, etc.). The alveoli are then filled with a mix of neutrophils, exudates and pathogens and this prevents the oxygen/carbon dioxide exchanges between blood and air normally present in the alveoli, causing respiratory distress and low oxygen pressure in the blood. Bacteria can spread in the body through the bloodstream and cause severe complications including meningitis, septicaemia, etc.
In the case of viruses, they invade the pulmonary parenchyma cells, destroying them. The response of the immune system (with lymphocytes) increases local damage and similarly to infections due to bacteria or fungi, respiratory distress and low oxygen pressure in the blood as a result of the effect on the alveoli. Viral infections of the respiratory tract can cause secondary bacterial infection by depressing the immune system.

- Pathogens

Bacteria: in humans, the most common is *Streptococcus pneumoniae*, but other bacteria that may cause pneumonia include *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Neisseria meningitidis*, or *Klebsiella pneumoniae*.

In Non-human Primates, *Streptococcus pneumoniae* , *Klebsiella pneumoniae* , *Bordetella bronchiseptica* , *Haemophilus influenzae* , and various species of streptococci, staphylococci, and pasteurellae can also lead to pneumonia.
Viruses: RSV, influenza, parainfluenza, adenovirus are the most common
Fungi: many fungi can cause pneumonia in humans, such as histoplasmosis, coccidioidomycosis, pulmonary blastomycosis and pneumocystis (these typically occur in immuno-compromised people (e.g. HIV Positive patients)), sporotrichosis (primarily a lymphocutaneous disease, but can involve the lungs as well), cryptococcosis and aspergillosis. Candidiasis can also in some rare cases result in pulmonary infections in immuno-compromised patients.
The fungi spores penetrate the lungs after inhalation or through the blood if there is fungal infection in another part of the body.
Parasites: in humans, these can yield ascariasis, schisostomiasis, toxoplasmiasis (caused by *Toxoplasma gondii*). The parasites enter the body through the skin or orally. They travel to the lungs usually through the blood. Parasitic pneumonia is rare in humans

- Symptoms

In Non Human Primates, symptoms include high fever, productive cough, sneezing, mucoid or mucopurulent nasal discharge, lethargy, anorexia, loss of weigh. The symptoms are more serious in bacterial pneumonia.
Especially in young individuals breathing difficulties can be serious and the general condition can deteriorate rapidly.

- Diagnostic
  - General examination
  - Pulmonary inspection: rales, sound variation upon percussion indicating consolidation of the lungs (they loose their "elasticity" because of the inflammation)
  - Blood count to see if there any signs of infection and to evaluate the state of the kidneys (important for the choice of medicine to be used for treatment of pneumonia)
  - Sputum examination and culture (if possible) to determine the pathogen and its sensitivity
  - X-Ray if available can be useful to confirm pneumonia (but not always) and see possible complications

- Treatment
  - **Antipyretic** and **analgesic** to reduce fever and pain (Acetaminophen, ibuprofen) (do not use Aspirin on young individuals because of the risk of Reye Syndrom – see Rosa Garriga's case study 2008)
  - **Expectorants** (carbocistein), **mucolytics** (Acetylcysteine) to help thinning and evacuation of mucus
  - **Give enough fluids to drink to prevent dehydration**
  - *If bacterial pneumonia confirmed or suspected*, **give antibiotics**: trimethoprim/sulfamethoxazole, amoxicillin, amox/clavulanic acid, cephalosporins (ceftriaxone IV if really severe case), fluoroquinolones (enrofloxacin)

- **Supportive care in really bad cases**: fluid therapy, oxygen therapy. You need to sedate the individual but do not use respiratory depressant anaesthetics!

➢ Prevention

The same rules as prevention of URI are applicable. But as pneumonia has a high morbidity and mortality rate, isolation of sick individuals is an important measure to prevent pneumonia from spreading in your sanctuary.

Remark: aspiration pneumonia is defined as the inhalation of either oropharyngeal or gastric contents into the lower airways. This leads to chemical damages of the lungs. It can cause severe respiratory distress and septic shock. Secondary bacterial infection is possible.
For treatment: intubation, aspiration of contents if possible, oxygen therapy, IV fluids, antibiotics (azythromycin, Augmentin©, ceftriaxone)

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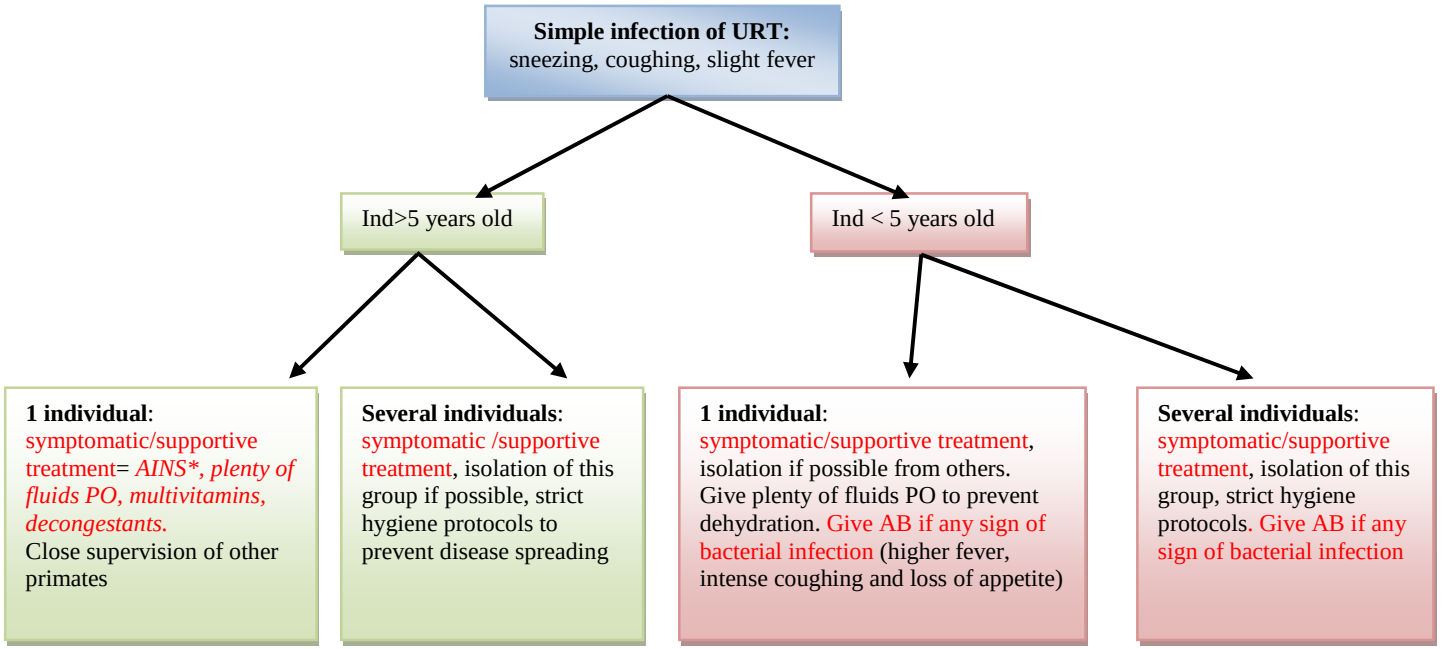
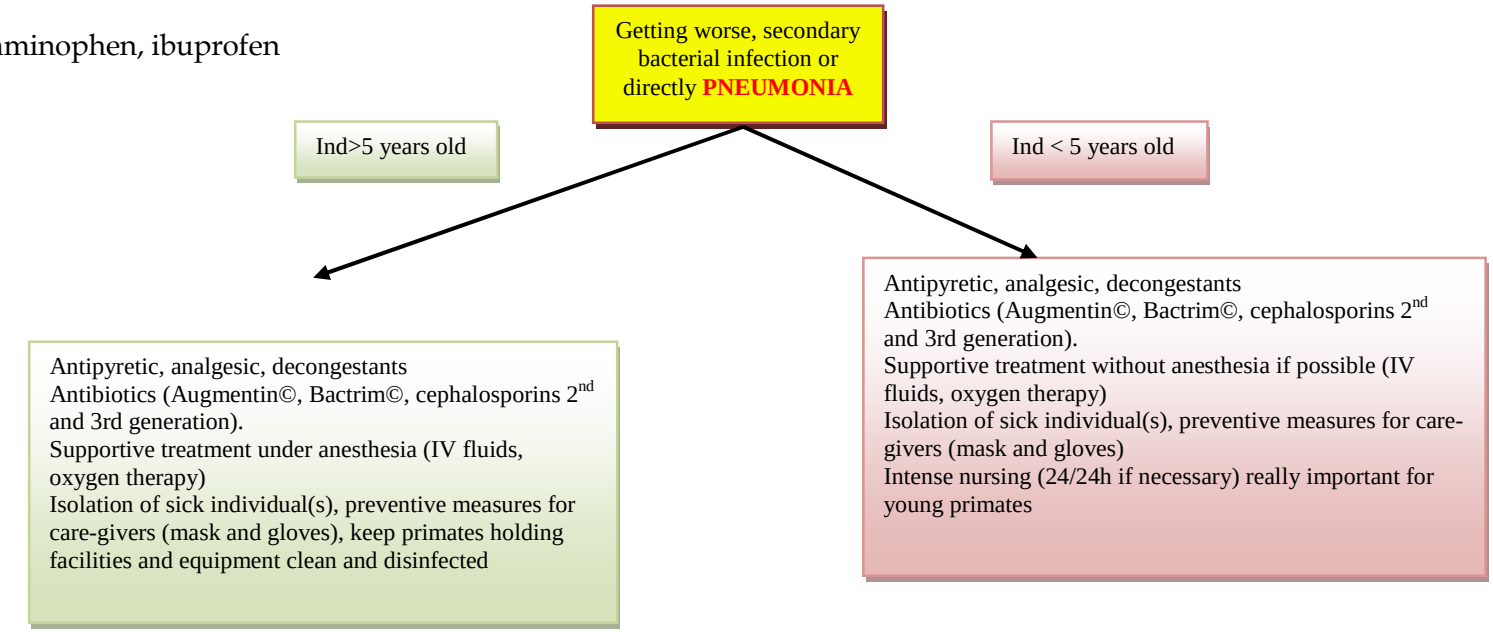


*AINS: acetaminophen, ibuprofen



4.3 TUBERCULOSIS and its CONTROL

L.Mugisha, S.Unwin and M. van Zijll Langhout

Tuberculosis (TB) is a major disease of concern in primates. Proper management requires constant testing and vigilance, as its presence can lead to severe clinical disease. Keys to control are accurate diagnosis, sanitation, health programmes for personnel, and quarantine.

PART 1. THE BIOLOGY OF TUBERCULOSIS

Background

Tuberculosis is a major cause of morbidity and mortality in adults worldwide (WHO, 2008). Currently, one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB). Annually, 8–9 million new cases of TB disease occur and more than 2 million people die worldwide (WHO, 2008) and 1.39 million people are infected with both with HIV and TB diseases (Corbett et al., 2003; WHO, 2008). The TB control programs face new challenge with emergency of multi-drug resistant TB(MDR-TB) in many countries and a recent epidemic of extensively drug resistant TB (XDR-TB) (WHO, 2008). Hence TB control strategies are constantly requiring improvement, including paying attention to potential reservoirs that might play a big role in emergency of resistant strains.
This current situation of TB worldwide, presents high risks of TB transmission to primates especially in captive facilities and quarantine centres where there are close interactions with human caretakers.

Transmission and Aetiology

Tuberculosis in humans may result from exposure to any one of the tubercle bacilli included within the *Mycobacterium tuberculosis* complex (i.e., *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. pinnipedii*, and *M. microti*). In non human primates it is a disease that is mainly contracted from humans by inhalation of infectious aerosols expelled from the respiratory tract of infected animals or humans with active disease. Transmission through ingestion is rare but has been reported (Sapolsky and Else, 1987). The occurrence of TB in non human primates is often caused by the same organism that causes TB in humans- *M. tuberculosis* - and the disease can spread rapidly from non-human primates to humans and vice versa. The second common type of TB, in macaques at least, accounting for about 15% of cases, is due to *M.bovis*, also a zoonosis, (Bernacky et al., 2002, CDC, 1993; Gibson, 1998 and Garcia et al., 2004). *Mycobacterium bovis*, unlike *M. tuberculosis*, has a wide host range, often isolated from tuberculous cattle, and has several wildlife maintenance hosts, including the Eurasian badger (*Meles meles*), brush-tailed possum (*Trichosurus vulpecula*), and white-tailed deer (*Odocoileus virginianus*). Wildlife reservoirs have made *M. bovis* eradication from national herds in several developed countries, including the United Kingdom, New Zealand, and the United States, particularly difficult . Eradication campaigns in these countries have generally relied on test and removal, slaughterhouse surveillance, movement restriction, and/or wildlife reservoir control strategies.

Although most species of primates are susceptible to tuberculosis, susceptibility varies. Old World monkeys are considered more susceptible than apes, which appear to be more susceptible than new World monkeys (Montali et al., 2001). Experimental studies of TB infection in three species of Old World monkeys revealed different levels of sensitivities to infection. African green monkeys (*Chlorocebus aethiops*) were highly sensitive to infection showing uniformly rapidly progressive disease; Rhesus macaques *(Macaca mulatta*) showed a more variable clinical course, while Cynomolgus macaques (*M.fascicularis*) experienced a more chronic course of infection (Mortzel et al., 2003).

Aetiology Summary

- Primate infection usually results from an association with humans
- *Mycobacterium tuberculosis* (most common), *M. bovis*, *M. avium-intracellulare* (least common)
- All primate species are susceptible

Clinical Signs

Clinical signs are usually chronic and vague unless entering the terminal stages of infection

- Irritability/ change in behaviour, al;so described in infected humans
- Wasting
- Enlarged lymph nodes or swelling of the abdomen
- Lethargy/exercise intolerance
- Anorexia
- Low grade fluctuating fever
- Sudden death
- Occasionally enlarged lymph nodes, draining tracts, disease of the spine (Pott's disease)
- Respiratory signs can be minimal. Even in vervet monkeys with gross lung lesions, coughing was rarely observed.
- When lungs are extensively involved, see dyspnea, intermittent, hacking cough. Abdominal breathing is more often observed than coughing.
- Neurological symptoms have been observed in a chacma baboon
- May be asymptomatic and shed organism
- Becomes generalized and progressive disease

Pathology

After primary infection, a range of clinical outcomes is possible from no overt disease, to rapidly progressive disease or more commonly, a chronic debilitating disease course. The general pattern of clinical outcomes following TB infection has been well described in humans into 4 stages of disease progression and resolution (Wallgren, 1948).
In the first stage, approximately 3 to 8 weeks after *M.tuberculosis* contained in inhaled aerosols becomes implanted in alveoli, the bacteria are disseminated by the lymphatic circulation to regional lymph nodes in the lung, forming the so-called primary or Ghon complex. At this time, conversion to tuberculin reactivity occurs. The second stage, lasting about 3 months, is marked by haematogenous circulation

of bacteria to many organs including other parts of the lung; at this time in some individuals, acute and sometimes fatal disease can occur in the form of tuberculosis meningitis or milliary (disseminated) tuberculosis. Pleurisy or inflammation of the pleural surfaces can occur during the third stage, lasting 3 to 7 months and causing severe chest pain, but this stage can be delayed for up to 2 years. It is thought that this condition is caused by either haematogenous dissemination or the release of bacteria into the pleural space from subpleural concentrations of bacteria in the lung. The free bacteria or their components are thought to interact with sensitized CD4 T lymphocytes that are attracted and then proliferate and release inflammatory cytokines (Kamholz, 1996). The last stage or resolution of the primary complex, where the disease does not progress, may take up to 3years. In this stage, more slowly developing extrapulmonary lesions, e.g, those in bones and joints, frequently presenting as chronic back pain, can appear in some individuals. However, most humans who are infected with TB do not exhibit progression of the disease.

Infected animals with active TB may show no overt signs of the disease for weeks or months, during which time they can transmit infection to other colony animals. However, as in humans, not all primary infections in other primates result in active TB disease and development of latent infections without overt disease is well documented (Capuano et al., 2003; Gormus et al., 2004). These animals with latent infections present a significant risk of reactivation and the development of active TB (Capuano et al., 2003; Flynn and Chan, 2001).

Although animals with latent TB are not infectious, reactivation of latent infections that were not deteced using traditional screening methods during primary quarantine is emerging as an important factor in the epidemiology of TB in nonhuman primates. This is a major reason why this disease is so difficult to control.

Focal granulomatous or miliary lesions in any organ are suggestive of Tuberculosis, but lesions are most regularly are found in the lung (Figure 1), liver (Figure 2), lymph Nodes (Figure 3) and spleen (Figure 4). In vervet monkeys the mesenterial lymph nodes were mostly affected. Secondary infection by swallowing the bacilli after coughing them up is common. In one case lesions in the kidney were found.

POST MORTEM EXAMINATION OF TB SUSPICIOUS ANIMALS

TB can affect all organs, but lesions are often hard to found. In early stages of the disease no macroscopic lesions are found at all. In case of TB suspicion it is essential to thoroughly palpate the lungs, liver and spleen for solid structures and to apply as many further diagnostics as possible to reduce a 'false negative'PM result. Necrotic abcesses can also be caused by other pathogens. Therefore always take fresh or frozen samples from the edge of lesions to confirm and identify TB infection by culture. Sensitivity testing is recommended to detect (highly) resistant strains, because these strains are very dangerous for humans.

Samples in 10% buffered formalin can be used to do histopathology and PCR.

When no macroscopic lesions are found, but antemortem tests have produced TB possible results, always take fresh or frozen samples from the mesenteric and mediastinal lymph nodes for culture. Prior to euthanasia take blood to store frozen serum for later analysis (Prima TB STAT-PAK or ELISA).

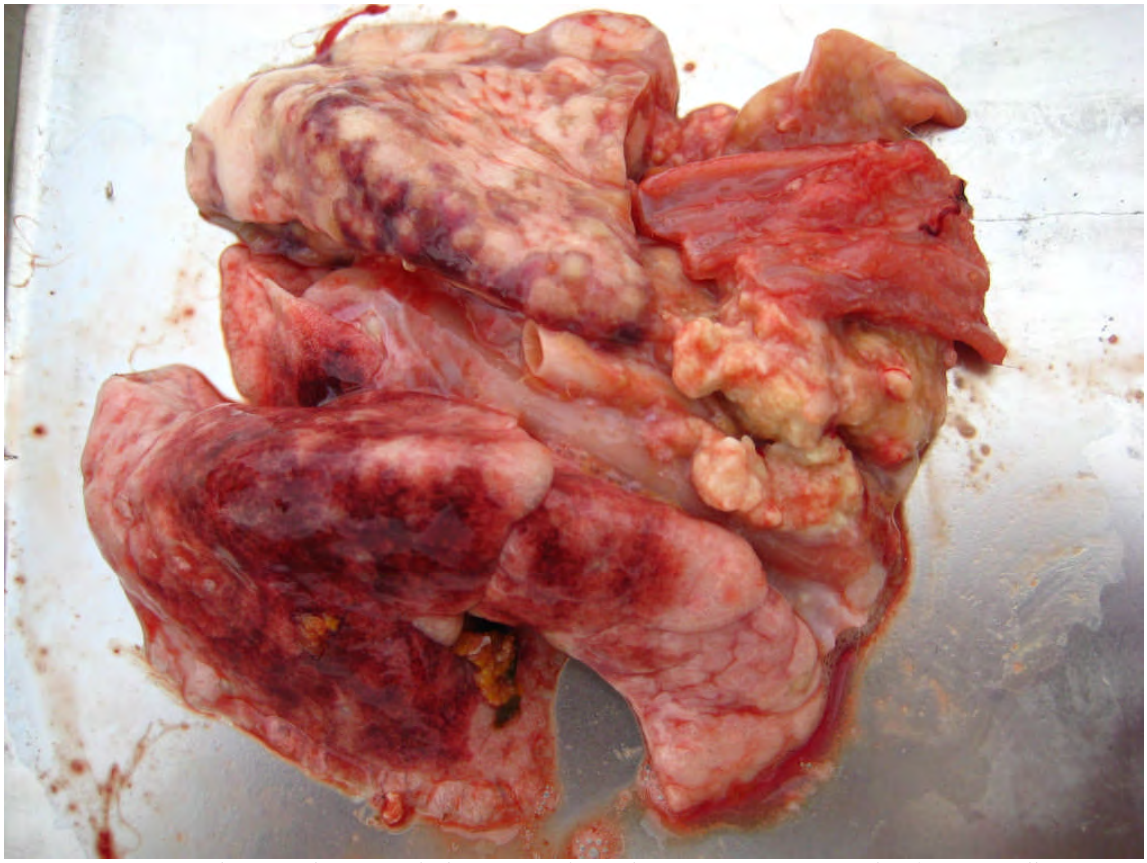


Figure 1. Granulomatous lesions in the lung in a Tubercuolsis case in a Vervet Monkey (M. van Zijll Langhout)

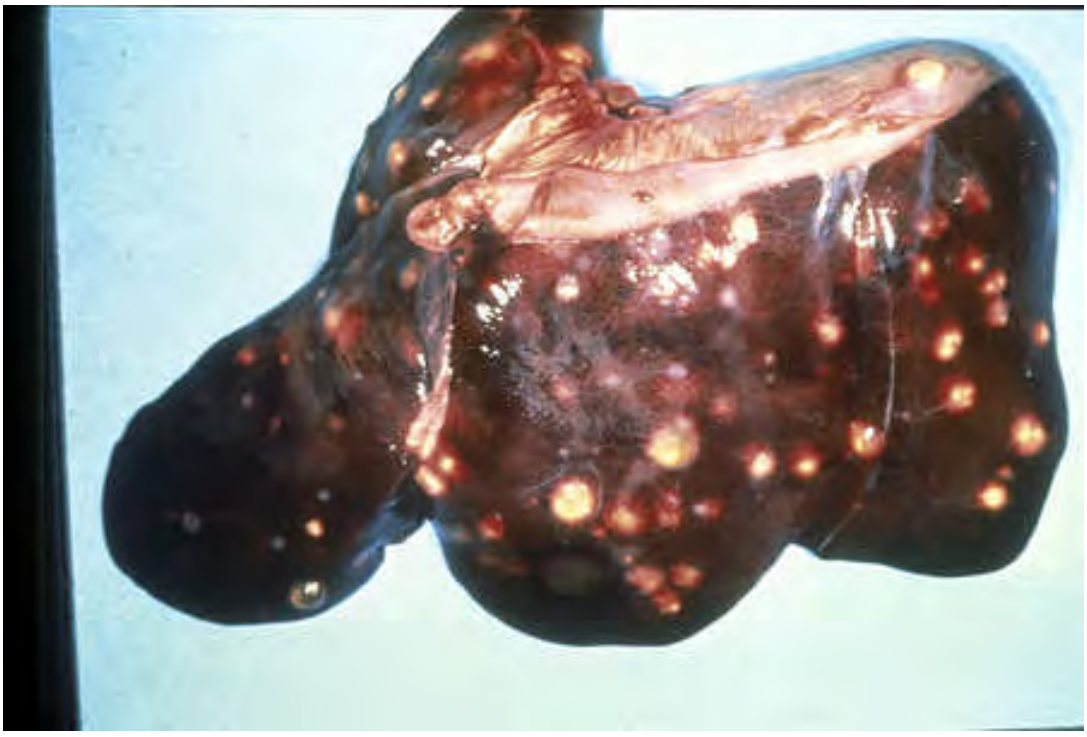


Figure 2. Milary Liver lesions in a TB positive Chimpanzee (L. Mugisha)

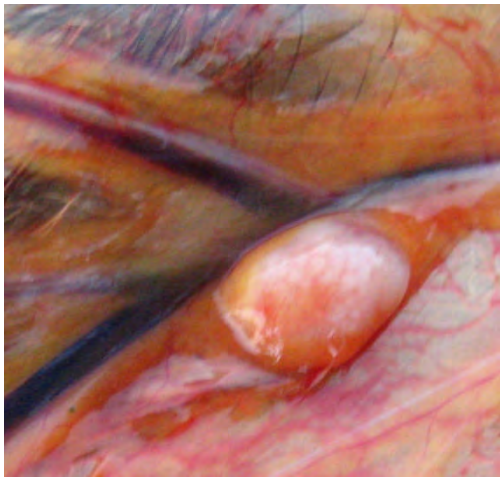


Figure 3. Enlarged Lymph Node in a TB positive Vervet Monkey (M. van Zijll Langhout)

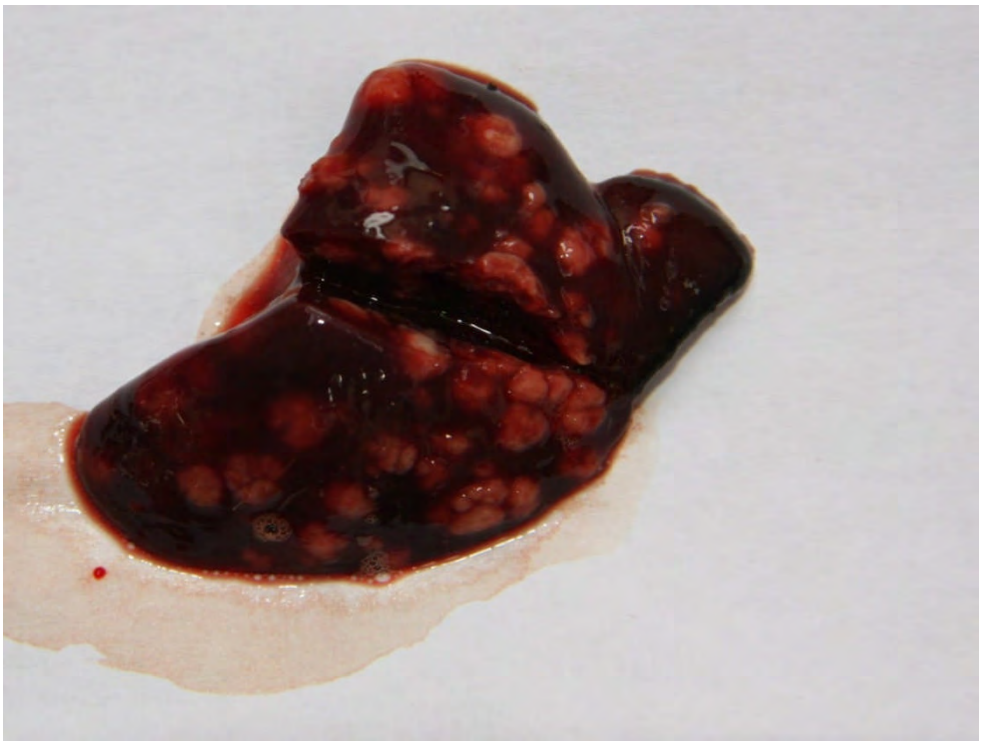


Figure 4: Granulomatosous lesions in a vervet monkeys, infected with M.tuberculosis (Photo: M.van Zijll Langhout)

PART 2. THE DIAGNOSIS OF TUBERCULOSIS

Table 1 below provides an overview of TB diagnostic techniques. In-depth notes on the various diagnostics techniques follow this table.

Table 1. Overview of TB testing

Testing Based on Detection of Mycobacterial Organisms		
Culture and speciation	8-12 weeks. Improved culture methods such as BACTEC, Septi-Chek, MB/ BacT systems and mycobacterial growth indicator tubes have the potential to reduce this time	Infection only found in 50% of human cases confirmed by other means
ZN staining	1 day	Other acid fast organisims such as Nocardia can produce false positives (reduced specificity)
Immunohistochemical staining	1 day	Labeled monoclonal antibodies may confirm acid-fast orgtanisms in tissues as being mycobacteria
PCR and MTb direct test (MTD) and RFLP (Restriction Fragment length polymorphism)	3 days	Can also do on fixed samples. More information required - may be used to distinguish pathogenic Vs atypical infections
Combination	Depends	In one study in deer, histopath had a PPV of 94%, acid fast 99% and PCR 100%
Secreted antigens (Ag85)	Depends	Requires more evaluation
Testing Based on Immunologic response to Mycobacteria		
PPD intrademal testing	72hr	PPV only 75% and then only in populations where prevalence is >10%
IFN-gamma (Primigam/ Bovigam/ cervigam/ quantoferon)	Hours if done on site, but requires precise conditions	Ancillary - assay to measure cytokine release. Specificity in buffalo 99.3%. Difficulties with using this test include (1) specific culture parameters need to be developed for each speciex (2) whole blood needs to be properly handled for accurate test results
ELISA	2-3 days	Most common serologic test. Incorporate various mycobacterial antigens for detection of antibodies. In cervids, specificity 78.6%, sensitivity 70% - VARIES WITH SPECIES AND ANTIGENS USED. Combine with PPD skin testing)

Multiantigen print immunoassay (MAPIA) - Chembio	24 hours	Specific antigens are printed as horizontal stripes on a nitrocellulose membrane. Strips can be cut out from this print out and incubated with test serum samples as a Western blot with an anti-immunglobulin conjugate anc colour developer. using this assay, an antibody response to mycobactetrial antigens is observed. Useful in a species where TB has a low prevalence, as appears more sensitive and can detect antibody as early as 4 weeks after infection
Immunochromatographic multiple antibody screening test (Chembio Stat-Pak) - Lateral flow technology rapid test	20 mins	Using selected mycobacterial antigens, RT was developed for rapid antibody detection that can use serum, plasma or whole blood. Usually used as a screening test for MAPIA. Sensitivity of 88.9%, specificity 98.5% (Chembio funded research)

Key to acronyms

PPV - positive predictive value = the proportion of all those who test positive who really are infected
PCR - Polymerase Chain Reaction
PPD - Purified Protein Derivative

No single test is 100% effective at diagnosing TB. Test specificity and sensitivity depends on stage of disease, type of mycobacterial infection and a host of other confounding factors. Readers are recommended to stay as up to date as possible, as new diagnostic techniques are developed regularly.

The main challenges in TB diagnostics has been lack of proper tools to detect latent and active TB, pulmonary and extra-pulmonary TB, anergy (overwhelming infection), TB in immunocomprimised hosts, immunologic cross-reactivity among mycobaterial species and for humans BCG-induced immune-reactivity.

A broad range of diagnostic tools have been developed over time especially in humans and some of the new approaches have been recently reviewed and are now available for the tuberculosis surveillance in Nonhuman primates (Lerche et al., 2008).

The diagnostic tools can be subdivided into

- Immune-response dependents such as Intradermal skin test, Gamma interferon tests or other cytokines tests, antibody detection tests and
- Tests that detect mycobacterium organism including culture of organisms, acid-fast staining of tissues, PCR or nucleic acid detection and immune-detection of mycobacterial specific antigens.

The tests most widely used for the detection of TB in humans and cattle include the measurement of delayed-type hypersensitivity (i.e., skin testing) to purified protein derivatives (PPDs) and/or in vitro assays for gamma interferon produced in response to mycobacterial antigen stimulation (i.e., Bovigam [Prionics AG, Schlieren, Switzerland] and Quantiferon Gold [Cellestis Inc., Carnegie, Victoria, Australia]). These tests rely on early cell-mediated responses, a hallmark of TB immunopathogenesis. Likewise, the intradermal tuberculin skin test (TST) using tuberculin purified protein derivative (PPD) and mammalian old tuberculin (MOT)

has been the main diagnostic tool of NHP tuberculosis surveillance and antemortem diagnosis for more than 60 years (Lerche, et al., 2008 review).

At this stage, for TB screening in primates in sanctuaries and under field conditions, we recommend a combination of the TST and Serological rapid test (StatPak), followed by increasingly specific tests if TB is suspected on the initial screening (Table 2). Prima TB should always be used in combination with the intra-dermal tuberculin tests. Both TST and Prima TB detect infection in different stages and therefore they are supplementing each other. Repeating the Prima TB in 10-14 days after the intra-dermal tuberculin test can in some cases show up positive individuals (Chembio). The higher level of antibodies as a reaction to tuberculin, increases the chance that the Prima TB detects them.

ID Skin Test[12]	Prima TB Statpak[13]	Thoracic Radiograph	PCR[14]	Culture[3]	AFB[15]	Recommendations
+	+	-	-	-	-	Strong suspect positive. Quarantine +/- Euthanase (once validated)
+	-	-	-	-	-	Quarantine. Retest in 2 months (full test range)
-	+	-	-	-	-	Quarantine. Retest in 2 months – OR *see MAPIA Protocol above if comes back online*
-	-	+	-	-	-	Test for other causes (infections, neoplasia) but quarantine in case anergic and retest if other cause not found
-	-	-	+	-	-	PCR is very sensitive – CONFIM WITH LAB – must be able to differentiate between M. tb complex and other mycobacteria. Quarantine for 6-12 months and retest.
-	-	-	-	+	-	Positive – Euthanase
-	-	-	-	-	+	Quarantine – Retest in 2 months
+	-	**If one or more of these are positive**			+/-	Positive – Euthanase
-	+	**If one or more of these are positive**			+/-	Positive – Euthanase

Table 2. TB Diagnostic suggestions and recommendtations under field conditions for Non human Primates.

[12] Mammalian Old Tuberculin (MOT) +/- Bovine PPD and Avium PPD comparative
[13] To be trialled in sanctuaries through 2010, to validate its use in this species as an improved screening test for TB (see above protocol/ recommendations)
[14] Conducted on Tracheal bronchial washes +/- Gastric lavage (10-30ML dependant on animal size)
[15] **Dr Wendi Bailey (Liverpool School of Tropical Medicine) is investigating a new, more specific AFB test utilising fluorescence. More information pending.**

1. The Intradermal Tuberculin Skin test (TST) and its interpretation.

The common method of monitoring TB in primates as approved by USDA is delayed hypersensitivity in response to an intradermal injection of mammalian old tuberculin (MOT) and comparative bovine and avian purified proteins into the skin of the eyelid (Kaufmann and Anderson, 1978).
The underlying principal of the TST is the detection of delayed-type hypersensitivity to tuberculin antigens. This has been the primary tool for detection of tuberculosis in primates since the 1940s and is currently the only ILAR/CDC-approved method for tuberculosis testing of animals in primary import quarantine (Kennard et al., 1939; NRC 1980; Roberts and Andrews, 2008). In NHPs, the delayed-type hypersensitivity to tuberculin antigens develops as part of the adaptive immune cascade within 3-4 weeks following infection. The protein fraction of the tuberculin is recognized by sensitized T- lymphocytes causing release of **lymphokines, local oedema and local cellular infiltration.** The amplitude of the hypersensitivity response and therefore the accuracy of TST readings may correlate with the number of (replicating) tubercle bacilli and is influenced by various factors including the amount of circulating, primed, antigen-specific T cells and the amount of specific antigen in the tuberculin preparation that is used for screening. Mammalian Old Tuberculin (MOT) is the most sensitive but least specific with 135000Tuberculin Units (TU)/ 0.1mL, Avium Purified Protein Derivative (PPD) is 25000 TU/ 0.1mL and bovine PPD has 1500TU/ 0.1mL. 1500 is at the very bottom of the range to illicit a response in non human primates. Human PPD, at just 5TU/ 0.1mL is the most specific but least sensitive and should NEVER be used in non human primates. Of the three tuberculins, Bovine is the least sensitive in picking up *Mycobacteria tuberculosis*, but in combination with avium, would at least be able to eliminate cross reactors. **We recommend Using MOT, as well as Bovine and Avian Tuberculin**

Composition

Tuberculin purified protein derivative (PPD) used for TST is prepared from culture filtrate of MTB and contains numerous antigens, most of which are homologous to vaccine strains of bacillus Calmette–Guérin (BCG), and environmental non-tuberculous mycobacteria (NTM). On the other hand, MOT is a poorly defined preparation composed of various mycobactaterial antigens that are known to be highly cross-reactive. MOT is less purified but contains more tuberculin units than PPD.

Method of TST delivery

- Carefully clean the eyelid of any dirt or debris with an alcohol swab, to prevent inadvertently injecting dirt and creating a local reaction separate to the tuberculin reaction looked for. Inject 0.1ml MOT intradermally into upper eyelid near the margin using a 27g needle, or a demarcated site on the abdomen (Ricther *et al*., 1994). The eyelid is a preferred site as it is relatively easy to observe in NHPs. The practice has been to use abdominal skin test when re-testing NHPs suspected of TB infection after the first TST. The abdominal skin test has an advantage in that the indurations can be easily measured, especially in very small species where the eyelid is difficult to use. However, there is accumulating evidence that the abdominal TST in NHPs may be significantly less sensitive and may

therefore be of limited utility in TB surveillance programs (Capuano et al., 2003; Motzel et al., 2003). In great apes, abdominal TST may not be of great value as it may not be easy to read as measurement often requires a further anaesthesia, an unnecessary risk.

- The batch number & expiry date of the tuberculin and which eyelid was injected should always be recorded.
- When using Avium and Bovine intradermally, by convention the Avium is injected intradermally into the right upper eyelid (0.1mL), and the Bovine into the left.
- If you are using all three, find an alternative site for the MOT that can easily be read (e.g flank or abdomen)
- Diagnosis is based on results of delayed hypersensitivity to intradermal tuberculin testing. The animals should be observed under good lighting for hypersensitivity reactions at 24, 48 and 72 hours post injection. The delayed hypersensitivity reaction will be most prominent at 72 hours, but the animal needs to be checked at 24 hour intervals, so local reactions are not misinterpreted as a positive delayed hypersensitivity. Any reactions or suspected reactions are to be observed and interpreted by the attending veterinarian. This is done according to established standardized scoring system for intrapalpebral reactions developed jointly by the California and Oregon Primate Research Centres; 0 to 5 grading system (Ritcher, et al., 1984) and abdominal skin reactions are scored on a similar 0 to 5 scale (Stanley et al., 1995).

Interpretation will be subjective, especially with an indefinite or suspicious result. In a known outbreak of TB in a population, grade 3 reactions should be considered TB positive. Table 2 and 3 should help with interpretation of the intradermal skin test.

Reaction at 72hrs	Grade	Interpretation
No reaction observed	0	Negative
Bruise only	1	Negative
Erythema without swelling	2	Negative
Erythema with minimal swelling, or slight swelling without erythema	3	Suspicious
Obvious swelling with drooping of eyelid and erythema	4	Positive
Swelling +/or necrosis with eyelids closed	5	Positive

Table 2. Interpretation of the tuberculin intradermal skin test

Score	Description	Interpretation
0	No reaction	**negative**
1	Moderate swelling, height of duration 3-5mm	**Negative**
2	Moderate sweeling, height of induration 5-10mm	**questionable**
3	Obvious swelling, height of induration <10mm	**positive**

Table 3. Interpretation of the tuberculin intradermal skin test when used on abdomen

Figures 4 to 6 demonstrates the TST procedure and positive hypersensitivity reactions to tuberculin, to assist interpretation

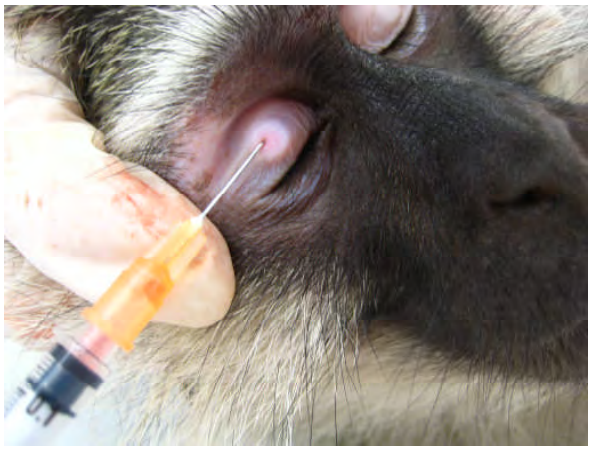


Figure 4: injection site for Avian Tuberculin PPD in the right eyelid (M. van Zijll Langhout)

Figure 5. Positive if see erythaema or swollen lid (Source, Internet)

Figure 6. Positive - complete closure of lid with necrosis and purulent discharge (Source, Internet)

Figure 7: Grade 5 TST reaction to Bovine Tuberculin PPD (H. Olbrecht)

Limitations

TST has a number of limitations with regard to sensitivity and specificity that reduce its ability to be used as a standalone test.

1. TST tends to give only intermittently positive results in a serial testing of infected animals(Garcia et al., 2004b; Walsh et al., 1996). TB infections were found in imported nonhuman primates to USA in 0.4% of cynomologus and rhesus monkeys of the 22,913 NHPs , representing 7% of the 249 shipments between 1992and 1993, CDC, 2003. All animals had received routine tuberculin skin tests (TSTs) (three tests, with 2-week intervals between tests) by experienced personnel in well-established quarantine facilities using accepted methods and a U.S. Department of Agriculture-licensed skin-test antigen.
2. TST has a tendency of giving negative results on TST-positive infected animals (even those with radiographic evidence of lung disease) on serial testing due to the development of latent infections, anergy associated with progressive disease or other mechanisms that are poorly understood (Heywood et al., 1970, Mayall et al., 1981; Motzel et al., 2003).
3. Lack of specificity of the mycobacterial antigens that constitute MOT, results in false positive TST reactions in animal sensitized to other, nontuberculous environmental mycobacteria (Brammer et al., 1995; Goodwin et al., 1988; Soave et al., 1981)
4. Unfortunately, the concentration of antigens required to elicit a positive TST reaction is higher in NHPs than in humans. MOT meets this requirement and has a greater reactivity than PPD and is therefore preferred to PPD as the regeant to use in a TST to identify infected animals. However, MOT is a crude culture of filtrate preparation that contains antigens common to many mycobacterial species including those not associated with TB. Because of this antigenic cross reactivity, TST suffers low specificity and false positive reactions are common.
5. Commercial production of MOT is cumbersome and hence there is only one manufacturer in USA (Synbiotics, Inc) and thus MOT is not readily available.

Outbreaks of TB continue to occur in established colonies of primates and can have severe consequences due to the loss of animals, transmission to human caretakers, disruption of re-introduction programmes and costs associated with disease control (Otto et al., 2004)). Detection of latent TB infections is therefore a high priority in the

control and prevention of disease in nonhuman primates. Limitations of TST described above and especially its inability to detect animals with latent TB infections, makes it an unsuitable tool to use as a single , standalone test for TB surveillance in non human primates. While the incidence of false positives and false negatives varies from batch to batch of tuberculin, false results have tended to be a problem with all skin tests aimed at diagnosing primate TB [Kaufmann and Anderson 1978, Walsh et al 1996]. None of the existing TB tests alone is sufficient to diagnose disease. Therefore, new TB diagnostic algorithms are being developed, in which serological assays may play an important role [Lyashchenko et al 1998,2000, Brusasca et al 2003]. One of these that shows promise, to be used in conjunction with the TST, is the Prima-TB Statpak, a rapid test lateral flow immunoassay.

2. Rapid Test Lateral Flow Immunoassay

The lateral flow assay utilizes a membrane impregnated with selected antigens combined with a sample pad, a conjugate pad, and a sink pad in individual plastic cassettes.

CHEMBIO'S PRIMA TB STAT-PAK®, FOR DETECTION OF TB ANTIBODIES
US Veterinary License No. 645
US Patent No. 2006057621
Distributed in North America by:
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Principle of Test
The PrimaTB STAT-PAK Assay is a qualitative, single use, two-step, immunochromatographic (lateral flow technology) screening test for the detection of antibodies to *Mycobacterium tuberculosis* and *Mycobacterium bovis* in primate serum, plasma or whole blood. The rapid test does not require laboratory equipment or specific technical training. This assay uses a"cocktail" of three immunodominant TB-specific antigens (ESAT-6, CFP-10, and MPB83), a combination that was sufficient to correctly identify 25 of 27 (93%) macaques (20 rhesus and 7 cynomolgus) experimentally infected with *M. tuberculosis* or *M. bovis*. Testing of 195 uninfected macaques produced 3 (1.5%) false positive results (Greenwald et al. 2007).
The test is used for the diagnosis of active tuberculosis (TB) in conjunction with other diagnostic methods. If specific antibodies are present in the sample, the expected test result is reactive. A reactive result is suggestive of active TB. In the absence of antibodies, the expected test result is nonreactive.
The test employs a unique cocktail of recombinant *M. tuberculosis* proteins that are bound to the membrane solid phase. Blue latex particles conjugated with protein are used as the detection system. Once a test sample is applied to the SAMPLE (S) well followed by the addition of a diluent, it flows laterally through the membrane strip. When it reaches the conjugate pad, antibodies, if present, bind to protein-latex

conjugate and then the migrating immune complex binds to the antigens on the solid phase in the TEST (T) area producing a blue line. In the absence of antibodies there is no line in the TEST (T) area. The sample continues to migrate along the membrane and produces a blue line in the CONTROL (C) area demonstrating that the reagents are functioning properly.
Results can be obtained in 20 minutes and require a small volume of serum, plasma, or whole blood.
The PrimaTB STAT-PAK Assay should be stored at 8 to 30oC in the original sealed pouch. The diluent should be stored in the original vial at 8 to 30oC. The kit is stable until the date imprinted on the box label and/or pouch.

Test Procedure
1. If test samples are refrigerated, remove them from the refrigerator and allow them to come to a temperature of 18 to 30oC before testing.
2. Remove the required number of PrimaTB STAT-PAK. Assay devices from their pouches and place the devices on a flat surface area. It is not necessary to remove the desiccant from the package. NOTE: If desiccant packet is missing, DO NOT USE, discard the test device and a new test device should be used.
3. Label test units with sample names and/or identification numbers.
4a. For Venous Whole Blood, Serum or Plasma: Using a laboratory pipette, obtain 30 uL of the specimen to be tested.
4b. For Fingerstick Blood:
Step 1: Prepare to perform the fingerstick blood collection procedure. Clean the finger with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze. Avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess fluid. Collect the sample from the second drop as explained in Step 2.
Step 2: Once the drop of blood has formed on the finger, hold the tube horizontally and touch the tip of the pipette to the sample. Capillary action will draw the sample to the black fill line and stop. Test immediately.
CAUTION! Filling is automatic. Never squeeze the pipette while sampling.
Step 3: To expel the 30 4l sample, align the tip of the pipette with the sample well and squeeze the bulb.
ONLY IF THE SAMPLE DOES NOT COME OUT OF THE TUBE, hold the pipette vertically and slide the finger over the vent hole. Then align the tip with the sample well and squeeze the bulb.
5. Once the specimen has been applied to the SAMPLE (S) well, invert the diluent bottle and hold it vertically (not at an angle) over the SAMPLE well. Add the diluent slowly dropwise; add 3 drops (~100 4l) into SAMPLE (S) well.
6. Read results at 20 minutes after the addition of diluent. Do not read any results after 30 minutes. Refer to INTERPRETATION OF RESULTS section below.
7. Discard the used disposable pipette, test device and any other test materials into a biohazard waste container.

Quality Control
A blue colored line should always appear in CONTROL (C) area if the test has been performed correctly and the device is working properly. It serves as an internal test procedural control. Good Laboratory Practice (GLP) recommends the use of control materials along with the test samples to ensure proper performance of the test kit. Positive and Negative serum or plasma based commercial controls should be used

for this purpose. Use controls as per the TEST PROCEDURE instructions of this insert.

Interpretation of Results

Nonreactive Result

One blue colored line in the CONTROL (C) area, with no visible colored line in the TEST (T) area indicates a nonreactive result. A nonreactive result at 20 minutes means that neither *Mycobacterium tuberculosis* nor *Mycobacterium bovis* antibodies were detected in the specimen. A nonreactive result does not preclude the possibility of TB infection.

Reactive Result

Two blue lines - one in the TEST (T) area and one in the CONTROL (C) area - indicate a reactive result (Figure 7). Intensities of the TEST and CONTROL lines may vary. Even a very faint line in the TEST (T) area of the device within 20 minutes is indicative of a reactive result. A reactive result means that *Mycobacterium tuberculosis* and/or *Mycobacterium bovis* antibodies were detected in the specimen

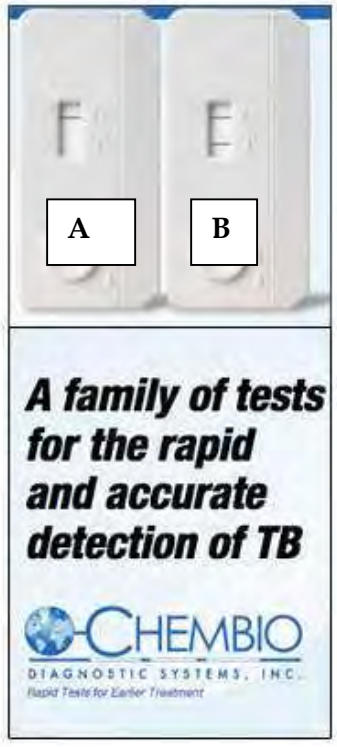

Figure 8. Comparing a non reactive (A) and reactive (B) test result for the Prima-TB statpak

Invalid Results

A blue line should always appear in the CONTROL (C) area, whether or not a line appears in the TEST (T) area. If there is no distinct blue line in the CONTROL (C) area, the test is invalid and should be repeated using a new device.

Limitations of the Procedure

1. The assay is designed for detecting antibodies against *M. tuberculosis* and *M. bovis* only from macaque plasma, serum or whole blood, although it has been used effectively in many other primate species, and is currently being validated in at least chimpanzees, gorillas, orangutans and vervet monkeys. Any result from the testing of other body fluids or of pooled serum or plasma samples should not be used.

2. A reactive result suggests the presence of antibodies to *M. tuberculosis* and/or *M. bovis*.
3. For a reactive result, the intensity of the test line does not necessarily correlate with the titre of antibody in the specimen.
4. Reading nonreactive results earlier than 20 minutes or any results later than 30 minutes may yield erroneous results.
5. Do not use hemolysed blood samples.
6. Be careful to add only 30 uL of specimen and 3 drops of diluent after applying the specimen to the SAMPLE (S) well.
7. Do not open the sealed test pouch until just prior to use.
8. Do not use kit contents beyond labelled expiration date.
9. Read results in a well-lit area.

P**erformance Characteristics of the Prima TB statpak, based on the manufacturers won trials, can be found in an appendix at the end of this chapter.**

One preliminary study in sanctuary chimps (Ngamba Island) using the PrimaTB statpak shows promise (Figure 8).

Status	Skin test result	Culture result	PrimaTB STATPAK	Necropsy result
Healthy (5)	Neg	ND	Negative	Neg (4)
Skin test+ (1)	Pos	M. tb	Positive	Pos
Skin test+ (3)	Pos	Cult pend (1)	Neg	ND
BCG vax (1)	ND	ND	Neg	ND

Figure 9. Comparing the Prima TB Statpak with Intradermal Skin Testing and TB culture results in a group of chimpanzees

Ngamba Island also did a small trial comparing the Elephant TB Statpak and the Prima TB Statpak results. Note that the Primate version correctly identified the TB positive chimp (Figure 9 (arrow), while the elephant kit remained negative.

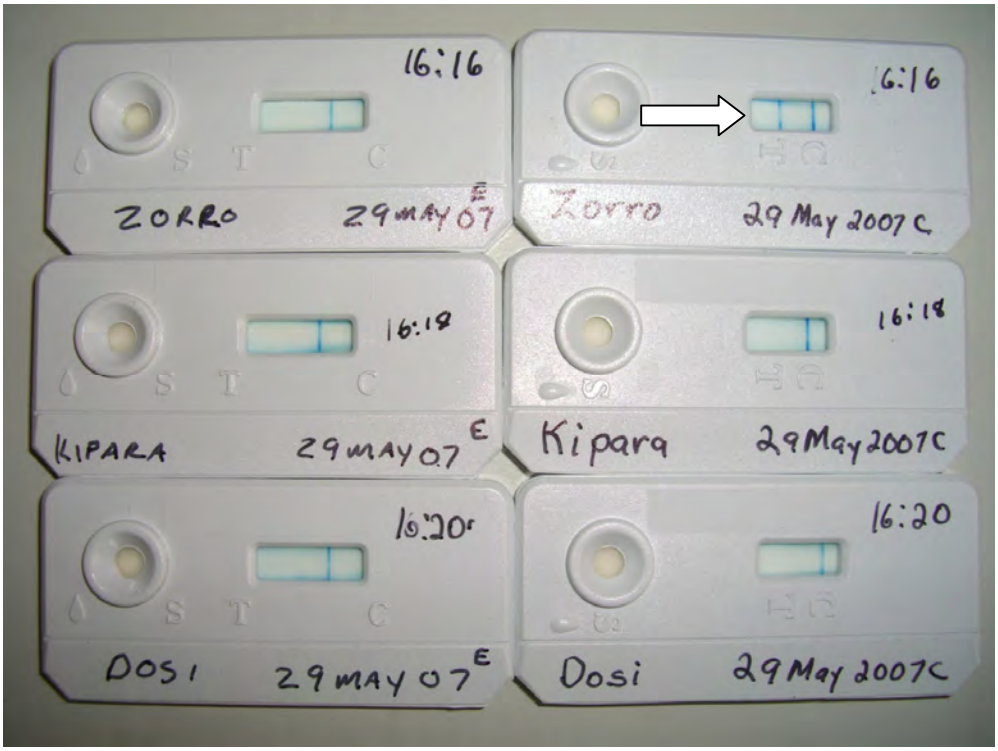

Figure 10. Comparing the elephant statpak (left) with the primate statpak (right). Note that the primate statpak correctly identified the infected chimpanzee (arrow), indicated the specificity of the pak. (Michelle Miller, 2007)

FURTHER DIAGNOSTICS (Provides information on other diagnostic tools especially applied in human TB diagnostics as a useful guide to new tools being developed for NHPs)

1. Thoracic radiographs and CT Scans

Chest radiographs may be used as an additional test procedure but cannot be used as the only screening procedure. A posterior–anterior chest radiograph can be used to detect chest abnormalities. Lesions may appear anywhere in the lungs and may differ in size, shape, density and cavitations. These abnormalities may suggest TB, but cannot be used for definitive differential diagnosis. Therefore, chest radiographs may be used as an additional test procedure only for screening TB. This additional test, however, is very valuable for the identification of animals that have a negative TST reaction because of immunosuppression associated with fulminant disease. Chest radiographs can be difficult to interpret and ideally are presented to an experienced radiologist or lung physician for interpretation. Because of the rareness of calcified tubercles, TB may present with a weak radiological contrast in non-human primates compared with other animals. There are no pathognomonic lesions, but enlargement of the bronchial lymph nodes may be an early sign of pulmonary mycobacteriosis. Larger tubercles or caviations may be appreciated radiographically.

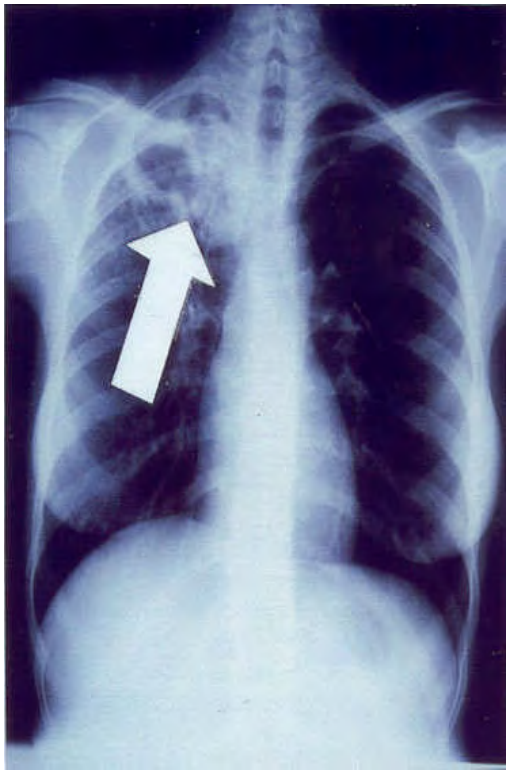

Figure 11. Lung Fibrosis in an advanced case of TB in a human

Infiltrates and pulmonary fibrosis can be seen in advanced cases (Figure 10).
Hilar lymph nodes are early site of disease (cardiac silhouette makes diagnosis difficult). However, changes are not specific to TB, and are similar to other diseases, such as pneumonia due to another bacterial infection.
Computed tomography scanning gave detailed real-time imaging of disease progression but is very expensive and not readily available.

2. Cytology and culture of bronchial wash.

This should always be taken at the same time as the initial TB testing (weather using intradermal testing, rapid test, or a combination). As culture can take up to 3 months, it is worth getting the sample as soon as possible, so if the screening tests do come up as reactive, the culture can be started sooner rather than later. Culture samples can be stored at room temperature for 7 days, allowing time to read the screening tests which will indicate if further tests are required.
The presence of acid-fast bacilli (AFB) on a sputum smear or other specimen may indicate TB. Acid-fast microscopy is easy and quick, but it has a limitation in that some AFBs are not *M. tuberculosis* (e.g *Nocardia spp*). Therefore, a bacterial culture is required on all initial samples to confirm the diagnosis. A positive culture of *M. tuberculosis* confirms a positive acid-fast staining and thereby the diagnosis of TB infection. Culture examinations should be completed on all specimens regardless of AFB smear results.
Culture of mycobacteria from clinical materials using Lowenstein–Jensen agar or other suitable cultivation media; gastric lavage with acid-fast cytology and culture of gastric mucus; tracheal or bronchoalveolar wash with acid-fast cytology and culture of tracheal mucus; faecal examination with acid-fast staining and culture; biopsy of altered organs with acid fast stain and culture and laparoscopy.

3. PCR

Polymerase chain reaction (PCR) can be used to detect mycobacterial DNA in any biological samples and intrinsically has the advantage of being much quicker than the conventional culture methods of diagnosis (bronchial wash and gastric lavage samples being the most useful). Detection of infection by screening faeces or sputum by PCR for mycobacterial DNA may therefore be considered as a rapid and alternative diagnostic tool for tuberculosis.

4. Interferon- γ Assays

IFN- γ is a critical cytokine in the cell-mediated immune response to tuberculin antigens, including the DTH response measured by the TST and in the host immune response to infection with tuberculosis mycobacteria (Collins and Kaufmann, 2001; Fletcher, 2007, Flyn et al., 1993; Lin et al., 2006). Hence assessing the IFN- γ response to TB antigens in vitro provides an alternative method for screening and diagnosis.
Recently a whole blood WB-IFN- γ based assay has become commercially available as PRIGRAM® TEST (Product number 63301, Prionics, Va Vista, Nebras ka, USA) , received provisional USDA licensure for use in nonhuman primates. The assay is based on the response of memory T-cells to stimulation with either tuberculin or TB-specific antigens, resulting in the release of IFN- γ. Briefly, within 24 hours of blood collection, 0.5 to 1.0ml aliquots of blood in 24-well culture plates are stimulated with a nil antigen control, purified protein derivative (PPD) of *Mycobacterium bovis* (bPPD), or *M.avium* PPD (aPPD). Although not included in the commercial version, some users of the assay add a mitogen-stimulated well as a positive control. After 24hrs of incubation at 37 oc and 5% CO2 in a humidified atmosphere, the concentration of IFN- γ in the supernant plasma of each aliquot is determined by enzyme immunoassay.
The interpretation of WB IFN- γ assay is based on the difference in IFN- γ response to bPPD and aPPD; the suggested criterion for a positive test is OD bPPD-ODaPPD ≥ 0.05 OD units. A positive reaction to bPPD is interpreted as an indication of sensitization to antigens of either *M.tuberculosis* or *M.bovis*.
Incorporation of the aPPD antigens addresses the specificity issue of cross reactivity among mycobacterial PPD antigens. A stronger response to aPPD than to bPPD is often
Interpreted as sensitization to *M. avium* or other non-tuberculous environmental mycobacterial species. This interpretation, however, should be based on test results from more than a single time point, as a small subset of nonhuman primates in the early stages of *M. tuberculosis* or *M. bovis* infection may show a stronger IFN- response to aPPD than to bPPD. Subsequent testing of these animals shows conversion to a dominant IFN- response to bPPD (Lerche, unpublished data; Vervenne et al. 2004). To avoid misdiagnosis, animals showing high avian PPD responses should be retested more than 2 weeks after the original test (Vervenne et al. 2004)
A whole-blood IFN-γ-test, QuantiFERON®-TB Gold (QFT-G; termed QFT-2G in Japan; Cellestis Limited, Carnegie, Australia), measuring cellular immune responses to two of these TB-specific proteins ESAT-6 and CFP-10, has recently been developed and received both US Food and Drug Administration and Japanese MHLW regulatory approval for use in humans. QFT-G-test has a high sensitivity and

specificity for diagnosis of active MTB infection, and importantly, is unaffected by BCG vaccination.4 More recently, it has been showed that QFT-G detects those likely to have latent TB infection (LTBI).(Arend et al., 2000; Scarpellin et al., 2004; Funayama et al., 2005; Haranda et al., 2006; Kang et al., 2005; Pai et al., 2005)

The ELISPOT Assay

New *in vitro* tests that are based on detecting the interferon-γ (IFN-γ) released by activated T lymphocytes (T cells),3 and whose antigens are a number of secretory proteins coded by the RD1 genes of the *M. tuberculosis* complex but are absent from the majority of environmental isolates, including BCG strains.4 Two of these proteins, early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), and synthetic overlapping peptides corresponding to the full length of each were initially tested and elicited a strong T-cell response in subjects with active TB infection or LTBI.5. The in-house assay is based on a restricted and highly selected pool of synthetic peptides containing multiple epitopes derived from ESAT-6 and CFP-10 proteins.(Brock et al., 2004; Diel et al., 2006).

Article III. The ELISPOT uses a known concentration of Ficoll-separated peripheral blood mononuclear cells rather than whole blood for incubation with stimulating antigens. The ELISPOT assay enables enumeration of the cells releasing IFN- by counting the "spots" of labeled IFN- through a stereomicroscope or automated ELISPOT plate reader (Lin et al. 2006; Wang et al. 2007). The ELISPOT IFN- assay has been used primarily for research purposes in experimental models, but a commercially available version (T SPOT-TB, Oxford Immunotec Ltd., Oxford, UK, commercial T-SPOT.TB™ assay7 and In-house ELISPOT-IFN-assay) has been licensed for use in human testing (Wang et al. 2007); to date, there is no commercial ELISPOT-based assay for use in nonhuman primates.

5. In vitro assays of humoral immune responses to TB antigens

Recent advances in the sequencing of the *M. tuberculosis* and *M. bovis* genomes (Cole et al. 1998; Garnier et al. 2003) have facilitated the identification of a number of secreted proteins that are unique to mycobacterial species of the *M. tuberculosis* complex (Amor et al. 2005; Harboe et al. 1996). Two of these proteins, ESAT-6 and CFP-10, are highly immunogenic and have been evaluated as antigen targets for detection of TB-specific antibodies in nonhuman primates. Gennaro and colleagues (Brusasca et al. 2003) reported that sera from 100% of 17 nonhuman primates from three species (cynomolgus, rhesus, and African green monkeys) experimentally infected with *M. tuberculosis* had detectable ESAT-6 antibodies, and 90% were reactive against two additional proteins, CFP-10 and-crystallin. Although animals tended to be positive by palpebral TST earlier than in the antibody test, the levels of specific antibody remained elevated over the course of infection, whereas TST reactivity was intermittent or waned (Brusasca et al. 2003).

In a naturally occurring outbreak of *M. bovis* infection in rhesus and cynomolgus macaques, 22 of 25 animals with tuberculosis lesions identified at necropsy had detectable immunoglobulin G (IgG) ESAT-6-specific antibodies, and the sensitivity and specificity of the antibody ELISA (88% and 84%, respectively) were comparable to the sensitivity (84%) and specificity (84%) of the TST (Kanaujia et al. 2003). Three confirmed positive cases in this outbreak did not have detectable antibody in early serum samples, and 5 of 32 animals (16%) without lesions were antibody-test positive (Kanaujia et al. 2003). Although seroconversion to ESAT-6, the earliest recognized antigen, occurs 1 to 2 months after infection, the persistence of detectable

antibody over the course of infection suggests that a significant improvement in TB surveillance programs may result from the addition of antibody testing for ESAT-6 and other specific antigens.
In addition to ELISA, several additional formats have been proposed for TB-specific antibody testing and may have applications for TB surveillance of nonhuman primates.

6. Multiantigen Print Immunoassay (MAPIA)

This assay allows for the simultaneous evaluation of the pattern of reactivity to multiple TB specific antigens is the multiantigen print immunoassay (MAPIA) (Lyashchenko et al. 2000). In this assay multiple antigens are immobilized in solid phase on nitrocellulose membranes as narrow bands using a semi-automated airbrush printing device (Linomat IV, CAMAG Scientific, Wilmington, NC). The antigen-coated nitrocellulose membrane is cut into 4 mm-wide strips that are then used for antibody detection by Western blot–like chromogenic immunodevelopment. Presence of a visible band is interpreted as a positive result (Lyashchenko et al. 2000). Use of multiple antigen arrays such as MMIA and MAPIA on sequential serum samples enables the assessment of changing patterns of immunoreactivity over the course of infection. This format can also serve as a confirmatory test for ELISA or rapid test reactive sera. In addition, multiple antigen arrays provide a powerful research tool for identifying novel immunodominant proteins, and may identify reactivity patterns that are predictive of disease progression or reactivation. MAPIA reactivity patterns have also been used to monitor response to therapy in animals undergoing treatment for tuberculosis (Lyashchenko et al. 2006).
Blood collection for Rapid Test (Chembio Stat Pak) and MAPIA. At 72 hours, repeat MAPIA (so needs another blood sample). Repeat the MAPIA again at 3 weeks. If there is an increasing antigen stimulation, this will be indicative of an active infection, as opposed to using the tests independently. NOTE – MAPIA is currently off the market (2009).

PART 3. GUIDELINES FOR THE PREVENTION AND CONTROL OF TUBERCULOSIS IN HUMANS WORKING WITH NON HUMAN PRIMATES. (to be read in conjunction with the relavent sections of the PASA Operations Manual and Section 1 of this vet manual)

Introduction

Tuberculosis is zoonotic with the potential to be transmitted from Humans to NHPs and Vice-versa with grave consequences in both scenarios, it is important to have guidelines for prevention and control of tuberculosis in sanctuaries managing primates and other captive facilities.

Personnel Prevention procedures

All individuals (including visitors) entering areas where non-human primates are housed may be at risk of acquiring *M. tuberculosis* infection or pose a risk of transmitting *M. tuberculosis* to non-human primates.

Step 1: All new staff should provide any past medical documents related to TB diagnosis and treatment if any. They should undergo baseline screening for M. tuberculosis exposure during the hiring process. TST or Rapid Test Lateral Flow Immunoassay (In-House ELISAPOT) and preferably a combination should be performed by the medical service provider chosen by the organization for easy follow up.

A positive TST or a history of a previous positive reaction to a tuberculin skin test should be followed by a chest radiograph.

A chest radiograph is obtained if:

a. the employee cannot provide documentation of a normal chest radiograph within the last three month following the discovery of the positive reaction, or

b. the employee's responses to the questions suggest active pulmonary tuberculosis, or

c. the employee did not receive appropriate chemoprophylaxis or treatment, as determined by the Health service provider

If there is clinical or radiographic evidence of active pulmonary tuberculosis, the person should not be hired to work with non human primates. He/ she should be advised/assisted to get medical attention. Conditions of hire after treatment should include provision of documentation establishing that there is no clinical or radiographic findings attributed to active pulmonary tuberculosis.

Step 11: Prospective employee with no history of a prior positive reaction to a tuberculin skin test will receive a ”two step” tuberculin skin test (Purified Protein Derivative, PPD test) and Rapid Test Lateral Flow Immunoassay on enrolment. The second tuberculin test should be given 1-3 weeks after the first. If the participant has documented negative PPD tests within the previous 6 months, only a single tuberculin skin test is administered.

Note: Persons who have received Bacillus Calmette-Guerin (BCG) immunization will be given PPD tests. Interpretation of a reaction will be based upon the size of the reaction, length of time since BCG administration, and risk of prior exposure to tuberculosis. A positive skin test may result from either exposure to *M. tuberculosis*, *M. bovis*, BCG injection, or exposure to non-tuberculous strains of mycobacteria. The

American Thoracic Society has published guidelines for the interpretation of intradermal testing. If inoculation with BCG occurred more than 10 years ago, a positive PPD test should not be considered a reaction due to BCG, but should instead be considered as positive for exposure to TB.
If the tuberculin skin tests are both negative and there are no other medical contraindications, the employee is medically cleared for work.

Step III: All employees should undergo annual tuberculin skin testing or medical screening for active tuberculosis infection. However, for persons working in quarantine and those working with suspected risky animals, the duration should be reduced to 3 to 6 months.

If the prior tuberculin skin test was negative, but the current test indicates positive, this is considered a recent conversion and the individual will undergo evaluation as in step one above. The employer should have a policy well documented on how to deal with cases TB acquired while on job as this is part occupational health and safety.
Employees with acid-fast positive sputum smears should be removed from animal contact until it is determined whether this represents infection with an organism of the *M. tuberculosis* complex (*M. tuberculosis* or *M. bovis*). Treatment guidelines and recommendations for contact with animals and humans are available through state public health departments.

Step IV: All volunteers and visitors likely to come into close contact with the primates, should present evidence of recent TB screening within the last three month either a TST or Chest radiograph before admitted into the facility.

Step V: Implementation
The resident Veterinarian/director has the responsibility to ensure all staff adhere to the TB screening guidelines and arrange follow up checks with each individual and medical personnel assigned with screening responsibility.
All entrants (including all visitors and volunteers) into non-human primate areas must be verified and certified free from of active tuberculosis.

Step VI: **Personal Protective Equipment (PPE) –** Items of clothing (i.e. lab coats, shoe covers, face masks, gloves, etc.) or equipment (i.e. face shields, eye goggles, etc.) designed to prevent or limit exposure to potentially harmful agents should be provided to staff throughout the entire period of employment.
Best practices for the safe conduct of work in biomedical and clinical laboratories and animal facilities in regards to *Mycobacterium tuberculosis* are listed in the 5th Edition of Biosafety in Microbiological and Biomedical Laboratories published by the U.S. Department of Health and Human Services in 2007. http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

PART 4. GUIDELINES ON THE PREVENTION AND CONTROL OF TUBERCULOSIS IN NON HUMAN GUIDELINES (also refer to the relevant sections of the PASA Operations Manual)

Preventive measures are required to protect NHPs from acquiring Mycobacterium tuberculosis complex (MTC - *M. tuberculosis*, *M. bovis* and *M. africanum*) through interactions with humans and other species. Non-tubercle forming, atypical mycobacteria species are also important but primarily because they may confound test results. Below are guidelines to follow.

A. Quarantine

All recently rescued primates must undergo a quarantine period of 90 days. During this period, three TST and/ or PrimaTB STAT-PAK Assay should be carried out. The first test should be performed 1 week after arrival and thereafter 6weeks interval for the rest of the tests. The reactions should be read at intervals of 24, 48 and 72 hours and interpreted as already described. It is important to accurately record the results of TST for each animal in additional to any other information that may be relevant like the social animal partners and any movements. A digital image and exact measurements of a suspect skin reaction is a useful tool to compare test results in repeated tests.

B. Post Quarantine Husbandry Practices

The animal husbandry and sanitation practices within sanctuaries should be designed to prevent the spread of pathogens including tubercle bacilli. Use of appropriate disinfectants is recommended. For rooms with primates with reactive TST , tuberculocidal detergent disinfectants (the label must read tuberculocidal – e.g 5% cresylic compound or phenol derivative such as sodium orthophenyl phenate) must be used in facilities housing NHPs. Periodically rotating the specific disinfectant to prevent anti-microbial resistance should be considered. Cleaning and other in-room equipment must remain in one room unless it is effectively disinfected between rooms. Sanitation schedules and practices must be in compliance with all applicable regulations, policies and guidelines. Husbandry practices must minimize the production of aerosols in animal rooms, e.g., sanitizing room surfaces and sanitizing animal cages and litter pans or trays. High pressure washing of cages and room surfaces can be performed only after the NHPs have been removed from the room and with proper protection of personnel including protection from splash.

Frequency of TST: The following intervals for testing of species or groups of NHPs is recommended during quarantine and post-quarantine holding. Because of a number of variables, the facility veterinarian may elect to test at less frequent intervals. When NHPs are tested at less frequent intervals than these recommendations, the facility veterinarian who is to receive any of those NHPs must be notified of that fact before the animals are transferred.

Species or Group	TST Schedule Quarantine as per NIH PM 3044-1	Recommended TST Schedule Post-Quarantine Holding
New World Monkeys	3 times, 6weeks apart	Semiannually
Macaque species	5 times, 6 weeks apart	Quarterly
Baboons	3 times, 6 weeks apart	Semiannually
Chimpanzees , gorillas and bonobos	2 times, 6 weeks	Annually
Patas	3 times, 6weeks apart	Quarterly
African green	5 times, 6weeks apart	Quarterly
Prosimians	3 times, 6 weeks apart	Semiannually

Special attention should be taken for anergic NHPs
Tuberculous NHPs infrequently become anergic to TST. Tuberculosis should be considered and further testing performed on animals that have unexplained weight loss or non-healing wounds. Additional testing may include: cytology and culture swabs of non healing wounds, chest radiographs, acid fast bacillus smear, culture and PCR (polymerase chain reaction) of gastric and/or bronchial lavage, PCR of faeces or tissues, and other methods as they are validated. Immunosuppression is known to interfere with cell mediated immunity and may interfere with gamma interferon production and TST results.

Managing Suspect NHPs: Tuberculosis should be considered and further testing performed on animals with a suspect response on palpebral or abdominal tests. Additional testing may include: testing the contralateral eyelid, performing an abdominal test if not already performed, chest radiographs, acid fast bacillus smear, culture and PCR of gastric and/or bronchial lavage, PCR of feces or tissues, in-vitro gamma interferon assay (Primagam®), antibody detection (ESAT-6 and CFP-10) and other methods as they are validated. The Chart below provides a guide on the steps to follow.It is recommended that initial screening for TB include the TST and Prima TB Statpak, with additional samples taken as is possible in each situation (see table 2).

Further comments on interpretation

- **A case with two questionable test results should be regarded as positive.**
- A negative reaction may indicate that no disease is present or that the disease has progressed to such an advanced state that the primates has become anergic
- A viral infection such as measles, a debilitating illness, corticosteroids, or immunizations may all depress sensitivity to the test.
- When retesting primates with questionable reaction observations should be made between 2 and 8 hours after the test is performed as well as daily,

since primates that are anergic may demonstrate a "flash" reaction, which quickly recedes.

- False positive reactions: Contaminated equipment, too frequent testing, sensitization to the adjuvant, trauma, cross reaction with other mycobacteria
- False negative reactions: Subcutaneous injection, anergy, steroid therapy, measles vaccines, inadequate dose, isoniazid therapy
- Suspicious reaction?
    - Isolate the animal
    - Retest after 60 days
    - Use a different site to where the positive reaction was shown
    - If positive to second test, animal should be considered infected

Handling Tuberculous NHPs

A. Immediate Euthanasia

When a clinical diagnosis of *M. tuberculosis* complex disease is made in a NHP, it is immediately euthanased and the carcass is taken for post mortem and histopathology, with associated further testing as necessary . The cage and room where the tuberculous NHP was held are sanitized and remaining NHPs are placed under quarantine.

Quarantine means:

1) access to the room is limited to a few essential personnel,

2) protective clothing (Tyvek® jump suit, shoe covers, head bonnet, mask, latex, nitrile, vinyl or rubber gloves and eye protection) is worn in the room and is not removed from the room except to be autoclaved,

3) other NHPs are not placed in or removed from the room, and

4) NHPs in the room are tuberculin tested every six weeks until five tests have been performed with negative reactions; the first of these tests is administered about one week after the test that identified the tuberculous NHP.

When 5 tests have been administered with negative reactions, the quarantine may be terminated, except that NHPs are not placed in or removed from the room until a tuberculin test is administered four weeks after the last of the 5 tests with negative reactions being observed. A diligent effort will be made to locate all NHPs that were housed within the last 60 days in the room in which the tuberculous NHP was housed. These NHPs will be tuberculin tested on the same schedule as the NHPs currently housed in the quarantined room.

B. Delayed Euthanasia

The euthanasia of a primate with *M. tuberculosis* complex disease can be delayed if the animal is of great value to a research project and can be isolated to minimize the spread of tubercle bacilli to other primates or humans. The room in which such a primate was held when the clinical diagnosis was made will be placed under quarantine as described. Most sanctuaries do not have required housing conditions to contain spread of tubercle bacilli, hence euthanasia should be carried out immediately.

C. Treatment of Tuberculous primates

As positive animals can never be considered disease free, usually elect for euthanasia. ANIMALS DESTINED FOR RELEASE SHOULD BE REMOVED FROM THE REINTRODUCTION PROGRAMME. DO NOT TREAT POSITIVE ANIMALS AND THEN RELEASE.

- Reserve treatment only for most valuable animals
- If decide to treat you need to understand the consequences
- Animal can never be considered free of the disease
- Reaction to skin test will be reduced
- May shed organisms after therapy is stopped
- Positive animal must be removed from all contact with other primates
- Vigilant against the possibility of transmission to other primates and humans
- Combination of isoniazid, ethambutol, and rifampin is recommended treatment
- Dose of isoniazid in great apes is 10-30 mg/kg per day
- May need to treat for 6-12 months
- Consult with experts, especially human physicians

1st Line of drugs	2nd Line	3rd Line	New drugs
Isoniazid	Streptomycin	Linezolid	Etambutol Congeners (SQ-109)
Rifampin	Cycloserine	Nebulised interferon-γ	Diarylquinolines (DARQ)
Pyrazinamide	P-Aminosalicylic acid		Nitroimidazole (PA-824)
Ethambutol	Ethionamide		
Rifabutin	Amikacin/Kanamycin		
Rifapentine	Cepreomycin		
	Levefloxacin		
	Moxifloxacin		
	Gatifloxacin		

Table 4 : Shows generation of drugs that have been used/being used in treatment of TB in humans

VI. Records

It is important that each NHP's tuberculin test be accurately entered into its clinical record. Facility records should include where the animal has been housed including dates. Accurate records are also important in detecting unexplained weight loss or non-healing wounds which may be indications of tuberculosis in NHPs.

SUMMARY

- TB continues to be a worldwide health threat for humans and animals
- TB in non-human primates is mostly transmitted from humans. Personnel health programmes and limiting contact by strict rules for visitors and staff are crucuail to prevent infection.

- Application of new diagnostic technology may allow more rapid and accurate detection of TB in non-human primates
- No diagnostic methos id accurate on its own and as many techniques as possible must be combined to reduce 'false negative' results.
- Availability and cost will continue to be major obstacles for diagnostic technology for sanctuaries
- Partnering with research, zoos, and industry may advance knowledge and tools available

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APPENDIX.

Performance Characteristics of the Prima TB statpak (Source, Chembio clinical trials).

Highly specific antibody binding proteins are used in the PrimaTB STAT-PAK Assay. The PrimaTB STAT-PAK Assay was compared to the standard USDA approved methods and the diagnostic performance was found to be superior.

Sensitivity and Specificity

Clinical trial studies were performed on 372 well-characterized samples to evaluate the PrimaTB STAT-PAK Assay. No cross reactivity was found in Rhesus macaques experimentally infected with *M. avium* or *M. kansasii* . The sensitivity of the PrimaTB STAT-PAK was determined by testing 106 samples. Of these samples 92/106 samples were reactive by the Chembio PrimaTB STAT-PAK antibody test kit (Figure 11).

	Infection	# of + results
Rhesus macaque	M. tb	45/55
	M. bovis	13/13
Cynomologus macaque	M. tb	15/16
	M. bovis	19/22
Total		92/106

Figure 12. Sensitivity trial for the Prima TB Statpak

The specificity of the PrimaTB STAT-PAK was determined by testing 266 samples. Of these samples 263/266 samples were nonreactive by the Chembio PrimaTB STAT-PAK antibody test kit (Figure 12).

	Status	# of neg results
Rhesus macaque	Normal	221/224
	M. avium or M. kansasii	6/6
Cynomolgus macaque	Normal	36/36
Total		263/266

Figure 13 Specificity trial for the Prima Tb Statpak

REPRODUCIBILITY STUDIES

Reproducibility was tested at three independent laboratories using three serials of PrimaTB STAT-PAK Assay. A reference panel of 20 blinded samples representing negative, weakly reactive and reactive were tested 3 different times on 3 different days. The compiled results from the 3 laboratories demonstrated 98.3% accuracy.

4.4DIFFERENTIAL DIAGNOSIS OF DIARRHOEA IN PRIMATES

W. Boardman (Reviewer S. Unwin)

Dietary – fruits,
Food poisoning
Chronic inflammatory bowel disease
Psychogenic

Shigella dysenteriae, flexneri mild to dysentery
Salmonella typhimurium
Campylobacter coli, jejuni,
Yersinia enterocolitica, pseudotuberculosis
E.coli
Clostridium difficile
Aeromonas
Vibrio parahaemolyticus

Entamoeba histolytica
Balantidium coli
Giardia
Other protozoa

Enterobius
Trichuris
Necator
Ancylostoma

Adenovirus – often respiratory disease too
Cytomegalovirus
Coxsackivrus
Rotavirus
Hepatitis A

DIARRHOEA CONTROL

What is diarrhoea?

- It is characterised by increased frequency and excess water content
- Leads to loss of large volumes of fluids which can lead to dehydration if intake of fluids is not adequate
- Leads to loss of electrolytes ie sodium, potassium and bicarbonate

Small bowel

- Large volume
- Malodorous
- Unformed/liquid

- Minimal mucous
- Loss of body weight

Large bowel

- Increased urgency and frequency
- Tenesmus (straining)
- Small volume
- Mucous
- Frank blood

Excess intake

- Excess fat
- Usually vomit

Maldigestive

- Decreased absorption of nutrients because of impaired digestion
- Pancreatic insufficiency
- Bile acid deficiency
- Following GI surgery
- Lactase deficiency

Malabsorptive

- Mucosal or sub mucosal disease impairing normal absorption
- Sodium transport mechanisms affected
- Exudation of blood, mucous and protein into lumen due to damagie ulcerative colitis

Secretory

- Increased secretion of water and electrolytes without damage
- Bacterial toxins
- Mechanical obstruction

CAUSES OF DIARRHOEA

Bacterial

- Campylobacter
- Salmonella
- Shigella
- E. coli
- Clostridial difficle
- Pseudomonas

Non Infectious

- Dietary – excess or incorrect food or hypersensitivity

- Stress/Psychological
- Food poisoning
- Inflammatory bowel disease

Parasites

- Strongyloides
- Trichuris - whipworms
- Enterobius – pinworms
- Necator - hookworms
- Ancylostoma - hookworms

Viruses

- Hepatitis A
- Rotavirus
- Adenovirus
- Measles
- Cytomegalovirus

Protozoal

- Balantidium coli → Common
- Entamoeba histolytica → Common
- Giardia lamblia → Common

Other

- Candida

TREATMENT

What to do first

Attempt to make a diagnosis

- History
- Clinical signs
- Faecal cytology
- Faecal culture
- Faecal wet prep and flotation
- Haemogram – if severe
- Electrolytes – if severe

Get a full history

- When started
- How many affected
- How depressed are the animals
- Characteristics of the diarrhoea
  - Frequency
  - Volume
  - Colour
  - Blood – severe sign

Take a stool sample and have it checked

- Examine to check consistency, content
- Wet preparation immediately to check for protozoa
- Faecal flotation for parasites
- Faecal cytology - stain with methylene blue to check for white and red blood cells
- Enterobius – use adhesive tape on perineum and applied to slide to check for eggs
- Culture for bacteria
- Observe the animals and check with primary care givers
- Weigh animals accurately – this is the best indicator of patients hydration
- Check demeanour, activity, appetite
- Check whole group
- Minimise contamination by isolation and regular removal of faecal material

Treat the cause

- Oral anti protozoals
  - Metronidazole
  - Tinidazole
  - Paromomycin
  - Secnedazole
- Oral antibiotics are useful if indicated:
  - Ciprofloxacin
  - Trimethoprim/ sulpha drugs
  - Gabrorral - aminosidine
  - Amoxycillin/ clavulanic acid
  - Chloramphenicol

If Mild – i.e. watery with no blood, behaviour normal

- Check faeces as soon as possible
- Feed only boiled rice, posho, human liquid supplements
- No fruit in particular and no formula of 24 hours
- Give oral rehydration fluids with a little cordial/ juice added to add taste
- Give probiotics to improve intestinal flora
- Treat the cause if there is one
- Give multivitamins
- Continue to monitor

If Moderate – i.e. watery with no blood, slightly depressed, appetite reduced

- Check faeces as soon as possible
- Feed only boiled rice, posho, human liquid supplements
- No fruit in particular and no formula for 24 hours
- Give oral rehydration fluids with a little cordial/ juice added to add taste
- Give probiotics to improve intestinal flora
- Start on antiprotozoals and or antibiotics
- Give multivitamins
- Continue to monitor

If severe and acute, often blood/mucous, appetite very poor, very depressed, often several affected.

Possible causes → Shigella, Salmonella, Entamoeba

- Check faeces as soon as possible
- Treat immediately with ciprofloaxacin and antiprotozoal / gabbroral – may need to give IV
- Oral – normally all that is needed in chimps. Use oral fluids little and often
- May need to resort to IV fluids – consider 10% dehydrated
- Start treatment – may need to sedate – very small dose of ketamine – 4-5mg/kg
- Give IV fluids slowly i.e. normal saline - 30ml/kg/hour for 2 hours under mild sedation
- Add bicarbonate at 1mEq/kg in 1:3 dilution with IV saline
- IV bolus of dextrose 5ml of dextrose 10% /kg over 15min
- Potassium can be added IV but extreme caution required
- Offer boiled rice, posho, human liquid supplements – consider naso gastric tubing
- Milk formula can be given after 48hours if drinking
- Give multivitamins

Prevention

- Good nutrition and good quality diets
- Regular faecal checks every 3 months, more frequent if continued problem
- Regular worming of whole group
- Minimise or discourage coprophagy
- Good staff hygiene
- Good enclosure hygiene and sanitation
- Effective quarantine system in place
- Control pests – insects, rats

4.5 DERMATOLOGY – SKIN CONDITIONS OF PRIMATES

C. Colin and W. Boardman

Skin diseases are common in primates. They are often seen in new arrivals at sanctuaries, or they can flare up in groups because of the contagious nature of many skin conditions. Some are zoonoses, so they can be transmitted to the staff, or the staff can transmit them to the primates as well! Skin disorders are often can be a sign of or secondary to a generalised problem. For example, immunocompromised individuals, either from another disease, or secondary to stress (e.g newcomers, dominancy issues or other behavioural issue in a group), will often show evidence of a skin disorder.
It's quite hard to detect primary skin lesions because they are too ephemeral to be observed, but they are really important for the diagnostic, as they are the direct reflect of the disease. So, when looking for skin lesions, take care and look cautiously, several times per day, or day after day to have a chance to find and identify primary lesions.

When dealing with skin disorders, prevention is better than cure so attempt to :

- manage new primate arrivals to your sanctuary to prevent potential spread of any skin condition being carried, (See section 1.2)
- have a good quarantine protocol, to prevent the new primate from transmitting any potential diseases to the rest of the primates in the sanctuary and to the staff (see section 1.2 and 3.4)
- have effective cleaning procedures of facilities and staff hygiene (see Section 1.2 and PASA Operations Manual)
- reduce the stress within the animals in the sanctuary as much as possible, including the risk of diseases transmission among the groups (refer to the PASA Operations Manual and the first 2 sections of this manual).

1. GENERAL EXAMINATION

Before concentrating on dermatologic examinations, start with a general examination, to see if there are other health problems, related to the skin manifestations:

- T°PR
- Mucosa: colour, lesions
- Examine every system (respiratory, cardiac, digestive, urinary, musculo-skeletal, nervous)

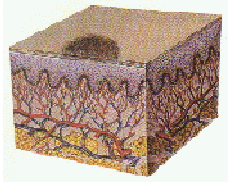
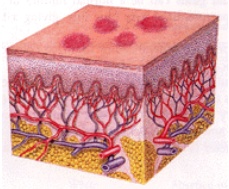
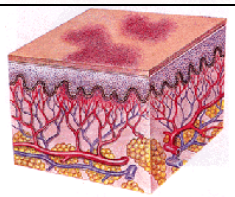
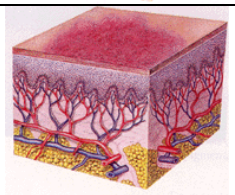
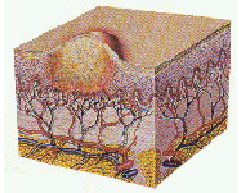
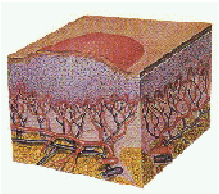
2. DERMATOLOGIC EXAMINATION

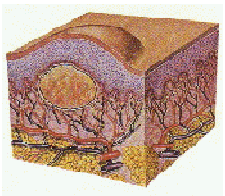
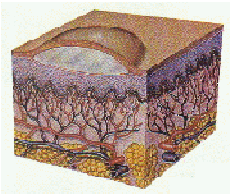
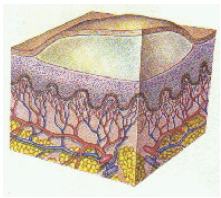
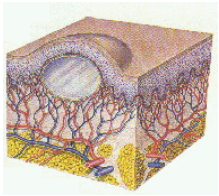
Several goals:

- To find cutaneous lesions
- To identify the lesions
- Localization and distribution of the lesions
- Check for ectoparasites

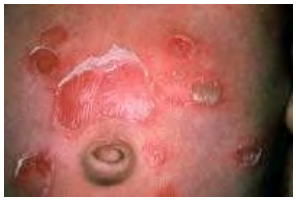
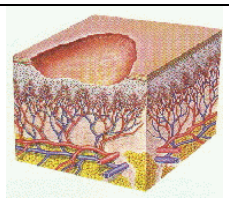
2.1 DESCRIPTION OF THE LESIONS: There are 3 kinds of skin lesions – primary, secondary or mixed. Skin can only show disease in a set number of ways. By correctly describing the lesions, you will have a better chance of arriving at the correct diagnosis quickly, and providing appropriate therapy

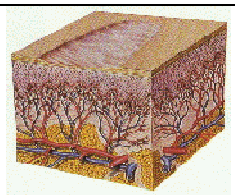
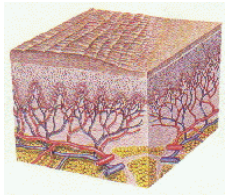
PRIMARY LESIONS: they are a direct reflection of a skin disease, they are ephemeral. They are really important to make a correct diagnosis

With colour change		
Macule	small, flat, circular spot, color changed (vitiligo, viral exanthem, drug eruptions) Several macules = **patch**	
Purpura	red or purple discolorations due to erythrocytes extravasations	
Petechia **Ecchymoses**	Punctiform lesion Large spots	
Erythema	redness of the skin due to capillary congestion (sign of inflammation	
The 'solid' lesions		
Papule	solid raised lesion. Several = **plaque.** Seen in allergic reaction (=wheal); Bacterial, fungal or parasitic infections for follicular papules; atopy or scabies for non follicular papules	 
Nodule	solid lesion more deeply rooted than a papule. Lipomas, fibromas, etc	
Tumor	bigger, abnormal growth or mass of tissue	

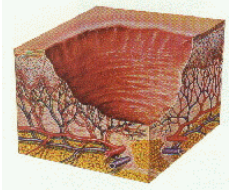
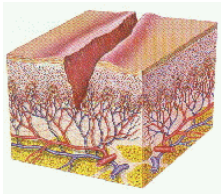
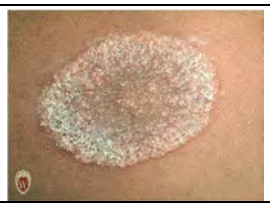
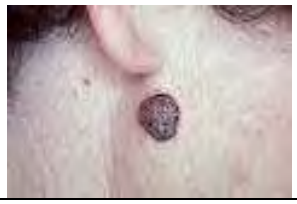
Cyst	cavity delimited by epithelium, that may contain air, liquid or semi/solid material	
Full of liquid		
Vesicle	raised lesion full of clear liquid. Large one = **bullae.** **Readily breakable lesions.** Insect bites, characteristic lesion of Herpes infection	 
Pustule	raised lesion filled with pus, results from an infection • Follicular pustules : bacterial, fungal folliculitis • Non follicular pustules : impetigo	

THE SECONDARY LESIONS: they result from a natural evolution of the primary lesions. They can be the result of a fungal or bacterial superinfection, or a consequence of scratching or treatment. These lesions are the most frequently observed, but they have little diagnostic interest

With abnormal production		
Epidermal collarette	more or less circular edge, formed by some or totality of the epidermis, which remains after the bursting of a lesion I (vesicle, pustule)	
Crust	dried collection of blood, serum or pus, consequence of rupture of lesion filled with liquid. Healing process	
With lost of substance		
Erosion	loss of epidermis	

Excoriation	erosion with traumatic origin, e.g. result of scratching	
With a continuous skin surface		
Sclerosis	indurations of the skin	
Atrophy	rarefaction of dermis components, giving the skin a thin and wrinkled appearance	
Lichenification	skin thickening, often with hyper-pigmentation, resulting from inflammatory process	

THE MIX LESIONS: they can be Primary or secondary.

With loss of substance or hairs		
Ulcer	deep loss of substance (dermis or hypodermis). **Fissure** if really deep	 
Alopecia	reduction of hairs density or lack of hairs	
With abnormal production of keratin		
Scale	dry, gray or whitish build-up of dead skin cells	
Keratoseborrhic state	dysfunction of cutaneous lipid production associated with keratinization troubles. Can be greasy or dry (e.g. ectoparasites)	

2.2 TECHNIQUES OF SKIN EXAMINATION

Skin scrape:

- Basic and easy examination
- provide information from epidermis, dermis
- To determine presence/absence of ectoparasites, fungus in dermis, in hair follicles

- For detection of mites, make several ones, in areas with papules/crust with a scalpel blade blunted
- Put collected material on a slide with lactophenol, examine under 10× as a scan, then at 40× to increase chance of correct diagnosis of any findings

Swab:

- In cases of otitis (ear infection), swabs can be used to collect material from the external auditory canal
- Put collected material on a slide
  - With lactophenol for *Otodectes* and other mites
  - roll the swab on a slide, staining to look for inflammatory cells and *Malassezia spp. (*fungus - yeasts)

Combing of the hair coat:

- To collect skin debris and cutaneous parasites including lice, fleas, ticks, some mites, etc.
- Collect these into a container or on a clean surface (see section 3.17 for storage)

Scotch (Cellotape) test:

- To detect ectoparasites. This is also used around the anus to detect pinworm.
- Paste the piece of scotch on a slide, and examine directly under 10× for detection of lice, 40× for dermatophyitis
- With staining for detection of *Malassezia sp.* under 100×

Cytology if available :

- To identify bacteria, fungi, inflammatory or neoplasic cells
- Impression smears on exsudative or ulcerating lesions
- Rupture pustules and vesicles with a sterile needle
- Stain slides and examine under 40× and 100× (oil immersion).

Others if available: biopsy, bacterial and fungal cultures, routine blood and urine tests, etc.

3. SKIN DISEASES

INFECTIOUS		NON-INFECTIONS
Ectoparasites	Scabies mite, lice, fleas, chigger, tumbu fly	**Nutritional disorders**
Bacteria	Impetigo, folliculitis, cellulitis	**Behavioral disorders**
Fungi	Ringworm, candidiasis	**Toxic**
Virus	Filoviruses, Herpes viruses, Pox viruses, Papilloma viruses	**Allergic**
		Neoplasic

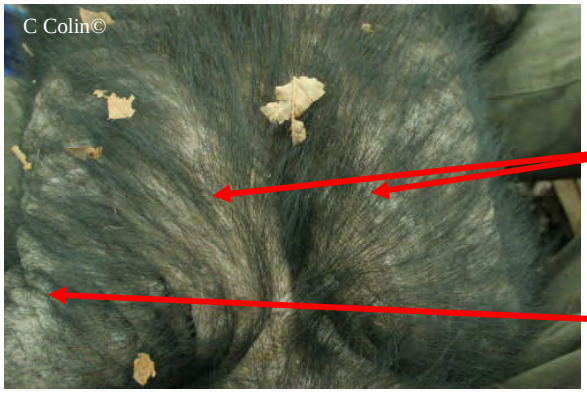
3.1 ECTOPARASITES

	Class	Ordre	Family
Scabies mite	Arachnida	Acarina	Sarcoptidae
Ticks	Arachnida	Acarina	Ixodidae ?
Chiggers	Arachnida	Acarina	Trombiculidae
Lice	Insecta	Anoplura	Pediculidae
Fleas	Insecta	Siphonaptera	Pulicidae
Tumbu flies	Insecta	Dipterae	Calliphoridae

Scabies

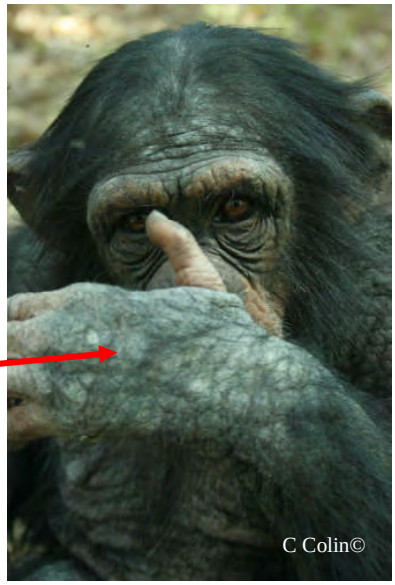
- Caused by mites, *Sarcoptes scabei*
- Characterized by superficial burrows, intense pruritus (EXTREMELY itchy) and secondary infections
- Spreads easily within a group, by skin to skin contact, holding facilities (indirect transmission)
- Potential zoonosis
- Adult female mite burrows into the outside layers of the skin
- Lays up to 3 eggs/day during her lifetime of 1-2 months.
- The development from egg to adult requires 10 to 14 days

- Scabies burrows hard to notice on skin of non-human primates
- Rash with itchy nodules (allergic reaction to the mites)
- Loss of hair, thickening and wrinkling of the skin and scab and crust formation.
- Secondary infection common, because of intense scratching
- Hyperkeratosis in extreme cases
- Can affect all the body



Symmetrical lesions

Hyper keratosis



Ticks

- Can transmit a lot of pathogens
- Crimean-Congo Hemorrhagic Fever (CCHF virus, fam. Bunyaviridae), a tick-born virus transmitted by *Hyalonema sp*. to wild and domestic animals, humans

- Rift Valley Fever (RVF virus, fam. Bunyaviridae) transmitted by *Aedes sp* but also by ticks.

Chiggers = Harvest mites (genus ***Trombicula***; also known as *red bugs*, *trombiculid mites*, *scrub-itch mites*, *berry bugs* or, in their larval stage, as *chiggers*)

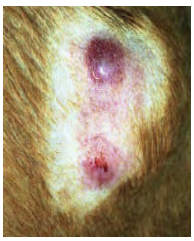
- Larvae feed on skin cells
- Post larval stage are not parasitic
- Responsible for severe itching, with papules and skin rashes, possible secondary infection due to scratching
- Do not spread from host to host
- Do not carry disease in Africa

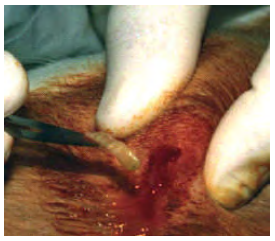
Fleas

- *Pulex irritans:* human flea
- *Ctenocephalides felis and P. irritans* found on monkeys
- Some fleas are really host specific, but most of them can feed on numerous hosts
- Can be vector of major zoonoses (plague, typhus)
- *Bartonellae quintana* found in *P. irritans* on a pet monkey (*Cercopithecus cephus*) in Gabon
- Monkeys can be infected by *Rickettsia* and be a reservoir as well as humans

Tumbu fly

- *Cordylobia anthropophaga:* "Tumbu fly" or "ver de Cayor" or "mango fly" or "skin maggot fly."
- Furoncular myasis due to larva of fly
- Eggs laid on the ground on dry, sandy soil often contaminated with urine and feces of animals. Eggs hatch after 1-3 days, larvae can wait up to 15 days for a host. Skin penetration really quick
- Evolution of 1-2 weeks, larva then emerges through the breathing pore and falls to the ground
- Small erythematous papule 2-3 days after larval penetration
- Evolution into a nodule which looks like a boil, with a pore in the center (where the larva breathes)
- Remove larvae manually (coating the breathing pore with a thick, viscous compound helps, e.g. vaseline)
- Surgery if necessary
- Great care when removing larva, rupturing larva might cause anaphylactic reaction
- Antibiotics recommended





Treatment

	Local treatment	General treatment	Treatment of environment
Scabies mite	Ascabiol, Spregal 2 or 3*/10 or 14days	Ivermectine 200µg/kg (up to 5 days) antibiotics	Good disinfection of resting and sleeping areas
Ticks	Manual removal		
Chiggers	Hot bath with soap	(Anti-Hi, antibiotics)	
Lice	Permethrin 1% 2*/7days	Ivermectine 200µg/kg	Clean
Fleas	Pet treatments?		Pyriproxifen or Methoprene repeated
Tumbu flies	Manual removal	Antibiotics	

3.2 BACTERIAL INFECTIONS

The natural defenses of the skin are composed of cutaneous endogenous flora, which are not primarily pathogenic, the physic-chemical properties of the skin and immunologic factors (immunoglobulins are secreted in sweat from Langerhans cells).

Inducing factors to a bacterial skin infection, include breakdown in one or more of these barriers due to poor hygiene, a skin alteration, a local or general corticosteroid therapy, ar use of other immunosuppressive drugs, or anything that can cause an immuno-depression. In many of cases, *Staphylococcus spp.* is implicated.

	Bacteria
Impetigo	group A beta-hemolytic *Streptococcus* (GABHS), and/or *Staphylococcus aureus*
Folliculitis	*Staphylococcus aureus*
Cellulitis	*Staphylococcus aureus, S. pyogenes,* in association with *Streptococcus* (and others)

Streptococcal infections = IMPETIGO :

- Highly contagious gram+ infection of the superficial layers of the epidermis.
- Due to group A beta-hemolytic *Streptococcus* (GABHS), and/or *Staphylococcus aureus* (most of the time association of both)
- Can be a primary infection or a surinfection
- 2 kinds : bullous and non bullous
  - **Bullous form**: Rapid onset of blisters that enlarge and rupture
  - **Non-bullous form**: Starts with a single eythemateous macule which evolves rapidly into a vesicle or pustule, ruptures leaving a crusted yellow exudate over the erosion

Staphylococci infections

- **Superficial folliculitis:**
  - Eruption of small pustules, each one around a hair
  - Do not confuse with dermatophytosis folliculitis
- **Deep folliculitis (furunculosis):**
  - Acute infection of a hair follicle due to *Staphylococus aureus*
  - Area can be hard, red, hot, painful
  - Fever and adenopathy possible
- **Cellulitis :**
  - Acute infection of the skin and soft tissue characterized by localized pain, swelling, tenderness, erythema, warmth
  - Caused by *Staphylococcus aureus* and *S. pyogenes,* but association with *Streptococcus,* and other bacteria !
  - Always a primary lesion (trauma, insect bite, or systemic disease like diabetes)
  - Other symptoms : fever, chills, myalgia, etc.

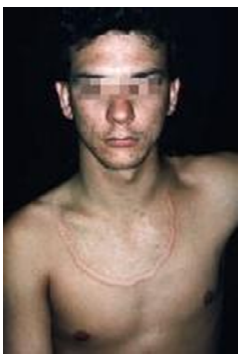
Treatment

	Local	General	Remark
Impetigo	Antibiotic creams 2-3 times/day for 7-10 days	Cephalosporines, semi-synthetic penicillin, If no improvement/resistance : tetracyclines,Bactrim©, clindamycin	Empiric treatment Make culture if possible when resistance
Folliculitis	Antiseptic, antibiotic creams	Dicloxacillin, cephalexin, Bactrim© for resistance	General treatment if bad
Cellulitis		Cephalosporines 1st and 2nd generations Macrolides Bactrim© Augmentin if allergy to macrolides And others	

3.3 FUNGAL INFECTIONS:

Ringworm

- Dermatophytes due to 3 genera : *Trichophyton, Microsporum* and *Epidermophyton*
- A large variety of dermatophytes (*T. rubrum* most common in the world)
- Zoonotic infections
- Environment, people, animals are carriers
- Can stay in environment : recurrent outbreaks can occur
- Direct and indirect transmissions
- Dermatophytes are in the cornified layers of the skin.
- Incubation period of 1-3 weeks
- Self-limited infection in healthy individuals but can widespread in young or debilitated animals
- Increased epidermal cell proliferation in response to infection, with resultant scaling.
- *Tinea corporis*: first lesions are eythematous, scaly plaques, and then formation of annular shape. Crusts, vesicles, papules often develop.
  - Not always itchy in animals
  - Dry lesions, red and circular, really typical



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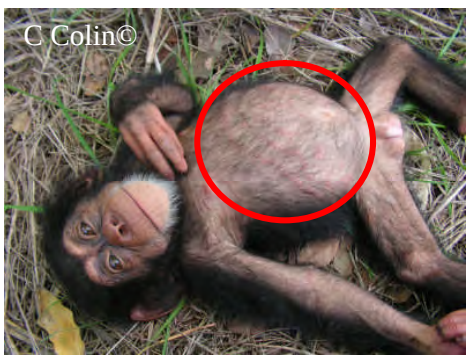

Tinea corporis in human and in a chimpanzee

Candidiasis

- Yeast, *Candida albicans*
- Superficial skin infections and mucous membrane
- A commensal fungus of gastro-intestinal tract of humans
- Affects immuno-depressed patients
- Induced by trauma, skin maceration, endocrine diseases, nutritional deficiencies (Fe, Vit B1, B2, B6, folic acid), low immune system
- Cutaneous forms in humans but NHP?
- Oropharyngeal candidiasis OPC = oral thrush, confirmed in chimpanzees:
    - OPC may clear spontaneously
    - Common in immuno-depressed infants
    - Pearly white patches on mucosal surface
    - Lesions may progress to symptomatic erosions and ulcerations
    - Can become systemic, but rare

Treatment

	Local	**Systemic**	**Remarks**
Ringworm	Topical azoles (*econazole, ketoconazole, miconazole, sertaconazole*, etc.) on the area and 2cm around, twice a day at least for 2 weeks	*Griseofulvin:* 10 to 15mg/kg/day for 6 weeks *Ketoconazol:* 3-4mg/kg/day but risk of hepatitis *Fluconazole:* 1mg/kg BID for 2 weeks	Give griseofulvine with fatty food to enhance absorption
Candidiasis	Nystatin Myconazole Clotrimazole topical cream	Fluconazole (Diflucan©) PO: *Adults* 200mg PO single dose or 100mg/day for 5 days (for thrush) *Infants* 3-6mg/kg/day 14-28 days	Do not treat OPC systematically

3.4 VIRAL SKIN INFECTIONS

Viruses are important pathogens in tropical areas; and many of them have muco-cutaneous manifestations. Viruses can spread quickly, mutate and cross species barriers. We now face the emergence of new viruses in Africa due to population growth and natural habitat destruction. Both Apes and humans can be efficient vectors for these viruses!

FILOVIRUSES

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- Ebola, Marburg viruses, responsible for severe hemorrhagic fevers.
- Ebola kills gorillas, chimpanzees and humans (and other wild animals)
- People can get infected by manipulating infected dead apes, meat, blood, etc.
- **In humans, characteristic, non puritic, maculopapular centripetal rash associated with erythema, which desquamate by day 5-7 of the illness.**
- Hemorrhagic manifestations develop at the peak of the illness, with ecchymoses, disseminated petechiae on skin and mucous membranes
- **Rash is characteristic in humans affection, for differential diagnostic**

HERPES VIRUSES

- *Herpes virus hominis* : HSV1, HSV2, EBV, CMV, VZV, etc
- Zoonotic
- Herpes B (*Herpesvirus simiae*), enzootic in rhesus, cynomolgus and other OWM of Macaca genus
- Like *Herpes hominis*, Herpes B characterized by lifelong infection with intermittent reactivations. Transmission among group through sexual activities and bites
- **Can be zoonotic, few human cases, but really severe (lethal in most of the cases)**
- In humans, vesicular skin lesions at or near site of inoculation + localized neurological symptoms, and at the end encephalitis
- In primates, no or mild symptoms

Simian Varicella-Zoster virus (SVV)

- Closely related`to Varicella-Zoster virus (VZV), which causes varicella only on humans
- Induces a varicella-like disease in OWM: fever and vesicular skin rash on the face, torso and the extremities (green and patas monkeys, also chimps and gorillas)
- Can produce a latent infection in neural ganglia and reactivate
- High morbidity and mortality
- Direct transmission by inhalation or direct skin to skin contacts

POXVIRUSES

- **Orthopoxviruses** :
  - Smallpox only on humans (NHP experimental)
  - MONKEYPOX (humans, NHP, rodents)
    - Orthopoxvirus with enzootic circulation in rain forests of central and western Africa
    - Zoonose, transmission by direct contact with an infected animal, indirect by eating undercooked meat, inoculation from cutaneous or mucosal

lesions of an infected animal especially with compromised skin barrier (bites, scratches, other…)
***In NHP (in captivity)*:**
- Multiple and discrete papules (1-4 mm diameter)
- **Lesions abundant in the palms of the hands, whole trunk and tail**
- Content of papules is very thick, similar to pus. Sometimes ulcerative circular lesions in the mouth.

- Parapoxviruses
- **Yatapoxviruses**: Tanapox virus and Yabapoxviruses (humans and NHP):
  - **Tanapox virus**
    - human cases in Kenya in the 1950's, and in the Sates in personnel of primate centers working with macaques
    - Nothing known about pathology in wild primates but studies showed that *Cercopithecus aethiops* could be a reservoir
    - In humans, skin lesions (nodules – papules – ulcers, then healing process)
    - In NHP in captivity, enlarged circles of skin with umbilication and adherent scab center. Lesions found primarily on the face (around the lips and nostrils) but also on other parts of the body.
    - Transmission by arthropods
    - **Yabapox virus:**
    - = Yaba monkey tumor virus
    - 1st case known in Yaba, Nigeria, in 1958 in a *Cynomolgus* colony
    - Causes subcutaneous tumor-like growth in monkeys
    - Lesions on the extremities, less frequently on the face.
    - Spontaneous regression of the lesions

- Papilloma viruses:
  - Responsible for warts
  - PV replicate on the skin or mucosal surfaces (mouth, genitals, anus, airways), in the keratinocytes
  - Transmission by direct contact via micro-lesions of the skin
  - Some PV lead to cancer but not frequent
  - Warts can be removed with surgery

3.3 NON INFECTIOUS SKIN DISEASES

a. Nutritional

Nutritional deficiencies are common in new arrivals at a sanctuary (see section 3.7, Malnutrition). In cases of malnourished animals (especially protein and vitamin deficiencies) you can see edema of the face, loss of muscles, associated with **hair loss**, **thin and rough dry hair**, **pigment loss**, associated or not with ectoparasites, ringworm, bacterial infections of the skin… and other clinical signs (intestinal malabsorption, leaver disease, etc.).

You must be careful with group living animals as there are always problems of dominance and of food access. Be sure that every single animal receive enough food, in quantity and quality at every meal. The food quality is a really important factor (See nutrition section 3.6 and its recommended texts).

b. Behavioral

This can be seen in different contexts in a sanctuary:

- Case of a new arrival: stress of previous captivity, of changes, new group….
- Stress among the group, bad dynamic in the group. Improvements are usually spectacular if you move the animal in another group where he will feel more at ease
- Auto mutilation behaviors in extreme cases of psychologic trauma
- Over self grooming if an animal get bored or is stressed. This can be improve with a change of group or by improving enrichment of the living conditions

c. Allergic reactions to plants (by contact or ingestion), caterpillars, insects…in the environment

- Intense pruritus with big papules. Usually, disappears in few hours
- Anti-Hi , corticoids by injection if it is really bad or on young animals
- **Be careful with generalized allergic reaction**, edema of the face and the throat = **emergency** = Quick edema (corticoids, epinephrine injectable). See Section 3.8 (Emergency Medicine)

d. Toxic

- **Snake bite:** pain, swollen, bleed, blister, necrosis at the bite site. General symptoms, like internal hemorrhages, nervous symptoms, muscle death, etc.
- Cleaning products: good use / kept in a safe place
- Cleaning protocol with the staff to avoid cleaning in presence of animals
- Topical treatments for ectoparasites, rinse carefully!

e. Neoplastic: it is really rare.

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Dermatology Checksheet
W.Boardman

Basic Principles

- Hygiene – direct contact reduction
- Zoonoses
- Moisture reduction
- Stress reduction
- Primary skin infection Vs. manifestation of systemic disease

Manifestations of Skin Disease

- A number of disease processes can be involved in any clinical sign
- Skin only has a certain number of ways in can present with disease
- Dry, flaky skin
- Oily moist skin
- Well circumscribed or generalised lesion
- Itchy or not itchy
- Raised lesion or flat
- Break in skin or intact
- Inflammation – reddened (increased blood flow to area), hot, painful, loss of function, swelling.

Non Infectious Skin Diseases

- Nutritional – see Nutrition discussion
- Behavioural – over grooming, bites (See Wounds discussion)
- Neoplastic – Spontaneous – very rare. Some initiated by viruses
- Toxic – cleaning fluid in satellite cages
- Allergies – usually very itchy

Infectious Skin Diseases

- Fungal – Ringworm
- Viral – Pox, Papilloma, Herpes
- Bacterial – see bacterial discussion
- Parasitic – Filiriasis – see parasite discussion.

Common Conditions

- Ringworm
- Dermatophytosis - zoonotic
- Fungus
- Direct contact, and via objects

- Carrier animals and in environment.
- Non-itchy, dry, red often circular lesions
- Juveniles more susceptible – if bad cases in adults – indicates underlying disease
- Associated with Vitamin A deficiency
- Treatment often not required especially if free ranging – but can last for several months.
- Miconazole (daktarin) – topical/ long term
- Griseofulvin – oral/ long term/ dangerous in females

HERPESVIRUS HOMINIS (SIMPLEX)

- Common, asymptomatic in humans
- Found in chimpanzees and gorillas
- Mild, self limiting oral vesicular lesions

MONKEYPOX VIRUS

- Original host = squirrel
- Lesions in chimps, gorillas and humans
- Direct contact
- Fever, rash.
- Human cases in Liberia, Sierra Leone, Zaire, Nigeria, Cameroon, CAR.
- Quarantine, Vaccine?

PAPILLOMAVIRUS

- Warts
- Direct contact
- Usually self limiting
- Can lead to tumours.
- Can surgically remove.

SIMIAN VARICELLA VIRUSES

- African Green Monkeys, Patas Monkeys
- Alphaherpesvirus – can be latently infected in ganglia
- High mortality/ morbidity
- Vesicles on skin – like chickenpox
- A chimpanzee and gorilla variant has been found – mild and self limiting
- More like a human form of the disease than the monkey form

SKIN MANIFESTATION OF SYSTEMIC DISEASE

e.g.

- Tip of the iceberg – gives an indication of deeper problems
- Examples only of what may see – not comprehensive
- DEEP PYODERMA

- Pain, exudate, sloughing of skin
- Fever, Depression, Anorexia
- FOOD HYPERSENSITIVITY
- Chronic itchiness, papules, pustules
- Diarrhoea
- SNAKE BITE
- Rapid and progressive oedema, pain, sloughing of skin
- Shock, coagulation problems, sepsis, respiratory compromise
- PROTEIN DEFICIENCY
- Scaling, pigment loss, patchy hair loss, thin hairs, rough dry hair, delayed wound healing
- Intestinal malabsorption, liver disease, starvation

4.6 OPTHALMOLOGY - EYE PROBLEMS

E Dubois. Reviewed by Nonee Magre

RED EYE

Introduction

The eye is an essential organ for animals living in the wild. For a binocular vision (estimation of distances), but also, living in hierarchic societies with constant risk of struggling for the animal's safety and security: an assymetric eye would be more attractive as a target! For the veterinarian this implies the best and the quickest diagnosis and treatment possible. Always try and put a diagnosis on an eye problem. Also, the infectious aspect for other animals must be considered. If in any doubt, I personally never hesitate to put an animal with an eye (s)' problem under sedation or anaesthesia for a better evaluation if the case.

Diagnostic Equipment

- Ophthalmic lamp
- Ophthalmoscope (if possible)
- Local anesthesic (drops"Novesin ®"0,4 % Ciba-vision)
- **Fluorescein Strips**
- To check corneal surface integrity
- To check lacrimal ducts drainage
- Schirmer Test (lacrimation test)
- Atropine drops 1-2 %
- Sterile swab for laboratory purposes (Transwab ® for aerobes and anaerobes) :
- Bacteriology – virology – antibiogram

Medication

NB: ***For a medicine with comparable components, I prefer ointments, which have a longer efficacy than drops !!***

Ophthalmic Anti infectives

Ointments include

- Bacitracin
- Choramphenicol
- Ciprofloxacin
- Gentamycin sulphate
- Idoxuridine Norflaxacin
- Polymyxin B
- Silver nitrate 1 %
- Tetracylcine
- Neomycin
- Tobramycin

Combination with corticosteroids

- Hydrocortisone /betamethasone /dexamethasone commonly combined with antibiotics and sulphonamides

No Steroidal antiinflammatory

- Diclofenac – sodium 0,1 % (Voltaren Opht)

Antibiotic Combination Products

- AK pol Bac - polymixin B and bacitracin
- Cortisporin - polymixin B, bacitracin, neomycin, hydrocortisone
- Isopto Cetapred - sulphacetamide-NA, prednisolone
- Maxetrol ointment - neomycin, polymixin B, dexamethasone
- Mycitracin Opht - choramphenicol, polymixin B, hydrocortisone
- Tobra dex - tobramycin, chlorobutanol, dexamethasone
- Vasocidin Opth - sulphacetamide NA , prednisolone

Miotics

- Ocusert pilo, Isopto carpine, Miocarpine- Pilocarpine

Mydriatics

- Atropisol, Isopto atropine - Atropine sulphate
- Mydriacyl - Tropicamide

Miscellaneous Ophtalmics

- Flourescein NA strips - Fluor-I-strip, Flourescein paper
- Schirmer test
- Lidocaine drops - Novesin
- Dorsolamide hydrochloride - Trusopt (ocular hypertension, open angle glaucoma)
- Timolol Maleate - Timoptic sol, Timoptic XE (ocular hypertension, open Glaucoma)

AETIOLOGY OF EYE PROBLEMS – CASE EVALUATION

Unilateral vs bilateral
Acute vs chronic

Therapy possibility

- Put drops-ointments
- Or daily treatments not possible?
- Substitution therapy? (Oral Antibiotics- Corticosteroides-Depot-Coticotherapy...)
- Repeated sedation / anesthesia for treatment?
- Traumatic
  - Injury
  - Fight
  - Foreign body
- Infections
  - Bacterial

- Viral
- Fungal
- Allergic
- Neoplasic
  - Metastatic
- Congenital
  - Hereditary
- Systemic
  - Diabetic cataract
  - Virus

So : aetiological + symptomatic treatment !

Differential Diagnosis: " Red Eye"

The following may all cause a "red eye"

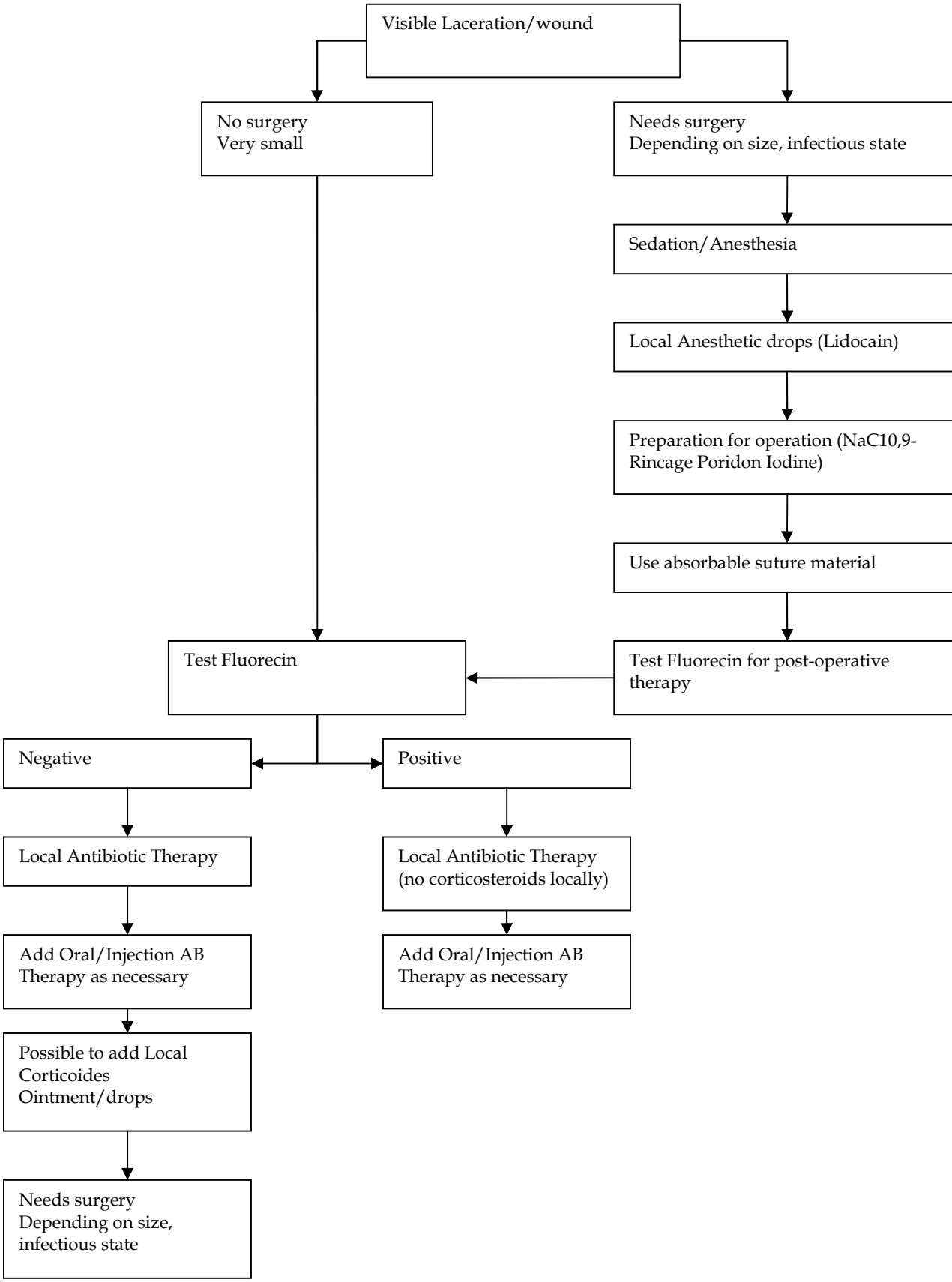
- Conjunctivitis
- Keratis (particulary ulcerative)
- Anterior Uveitis
- Glaucoma
- Retrobulbar abcess
- Retrobulbar tumor
- Scleritis /Episcleritis

Clinical Signs	Condition			
	Anterior Uveitis	**Conjunctivitis**	**Superficial Keratitis**	**Glaucoma**
Conjunctiva	Not thickened	Thickened hyperemia	Not thickened	Not thickened
Conjunctival Blood vessels	Circum corneal	Diffuse	Diffuse, tortuous	Diffuse superficial
Discharge	No	Yes	Yes,Serous –Mucous-Purulent	No
Pain	Moderate	No	Moderate or important	Severe
Photophobia	Moderate	No	Important	Moderate
Cornea	Clear or oedema	Clear	Opaque	Oedema
Pupil	Small, irregular	Normal	Normal	Dilated

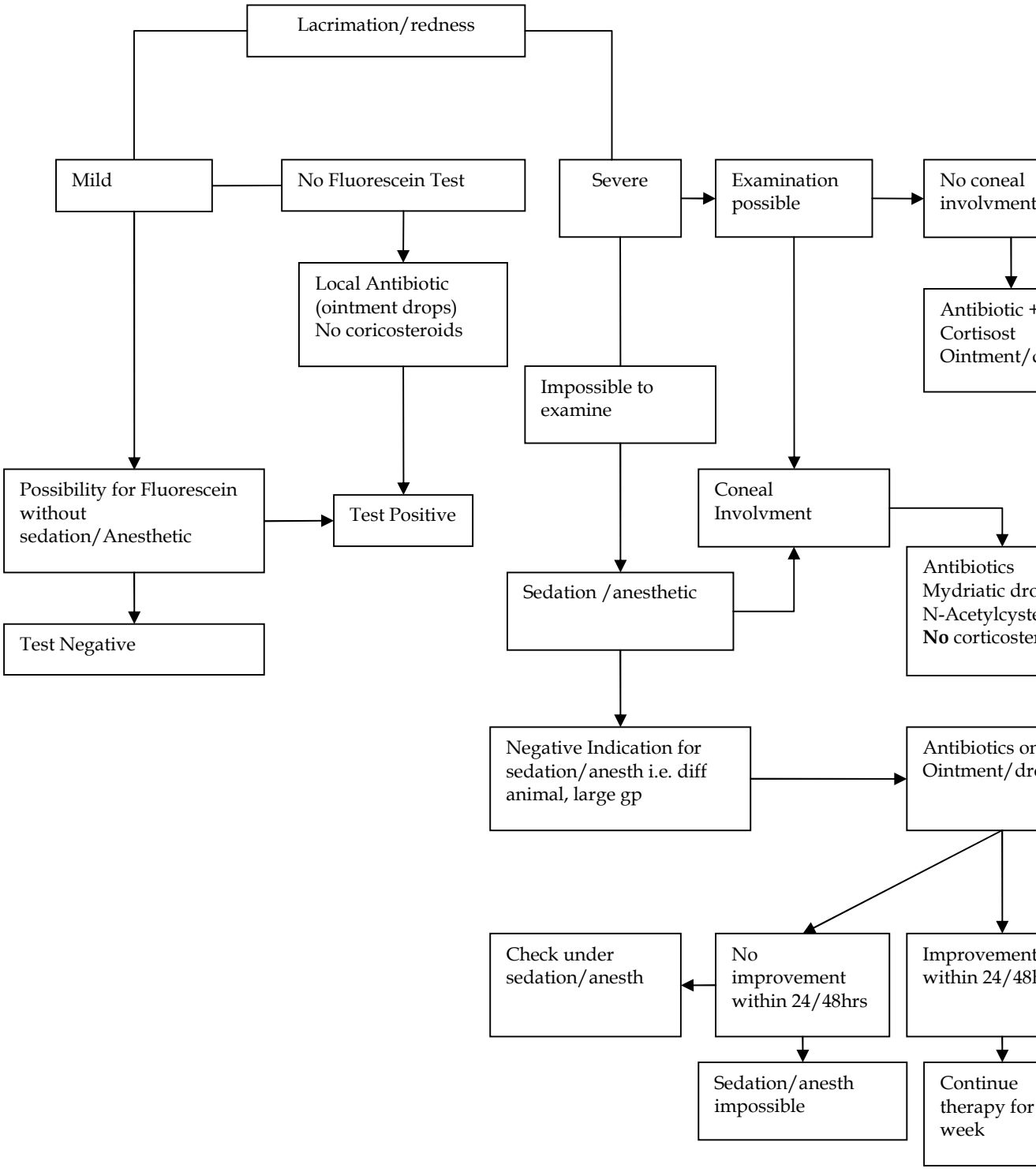
Pupillary light reflex	Decreased	Normal	Normal	Decreased-poor-absent
Ocular pressure and tonus	Lowered	Normal	Normal	Raised

Methodology - Flow Charts A-D are shown below

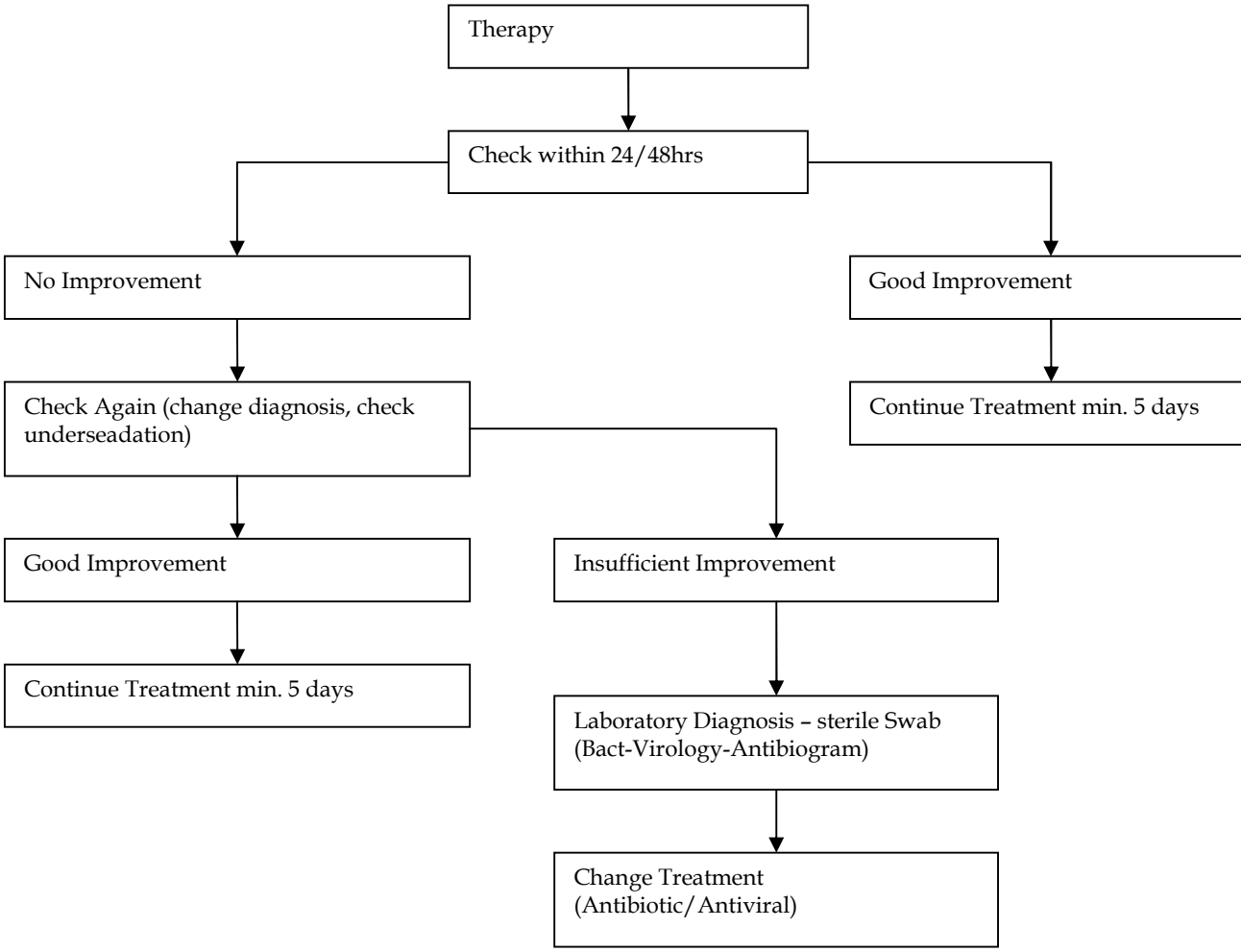
A1 UNILATERAL EYE PROBLEM



A2 UNILATERAL EYE PROBLEM



B **Therapy : Follow up**



C **Special case and treatment : keratitis superficialis chronica**

- Signs: Corneal Infiltration- Vascularisation- Opacity Exsudate
- Test Fluorescein = Negative !!!
- Therapy : Long term corticosteroid ointment or drops (BID – TID)

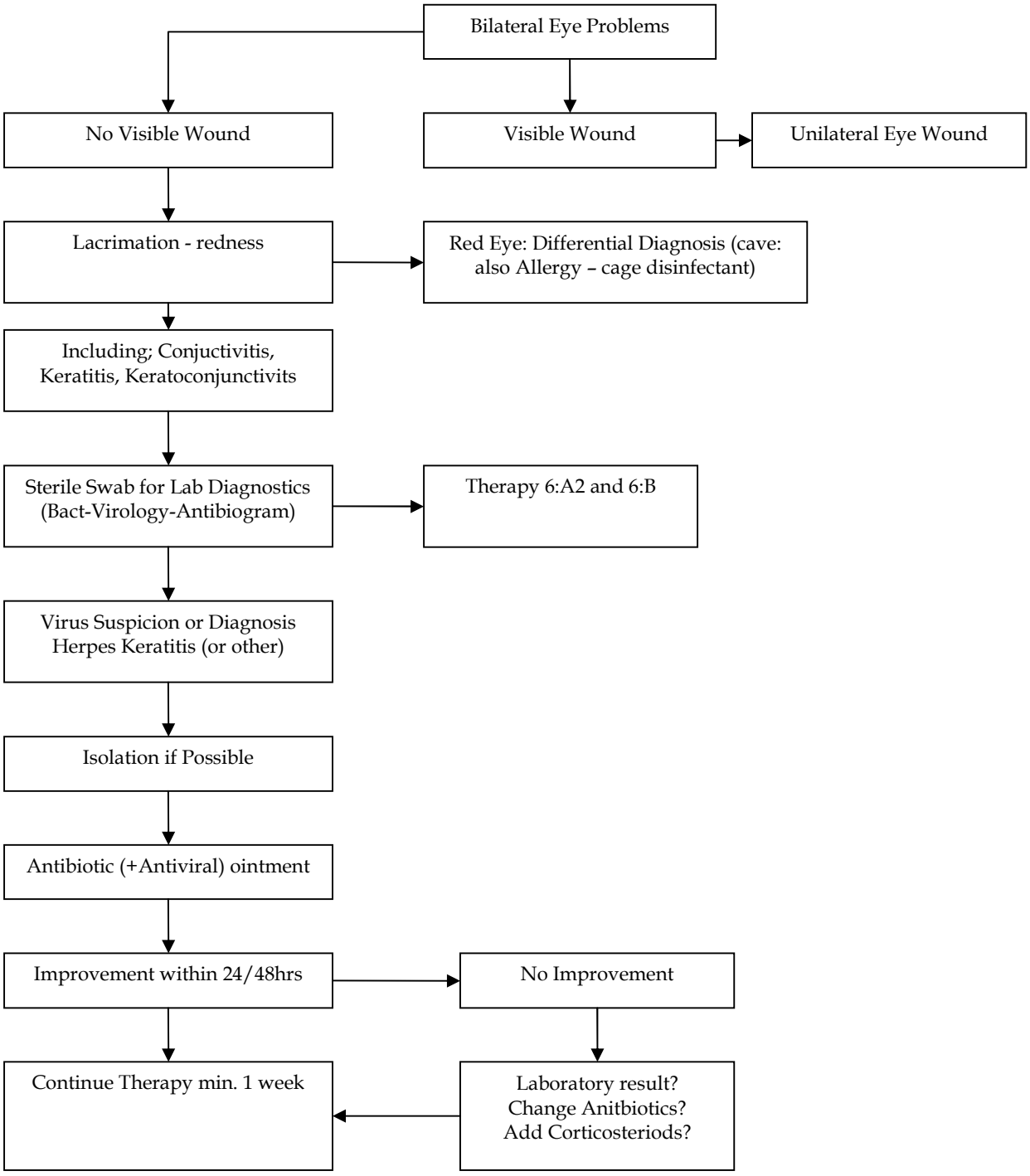
!!!! Alternative Therapy subconjunctival depot-corticosteroids (0,3-0,4 ml) injection under sedation - repeat every 4-6 weeks as necessary

- Add Antibiotic ointment if mucous or muco- purulent discharge (min. 5-7 days)

D Bilateral eye problem

If two or more individuals have signs of infection (lacrimation – redness ...)

- Always try to remove conjunctival secretions on a sterile swab for laboratory diagnosis.
- Try to isolate infected (infectious ...) animals.
- Caregiver should be instructed on good isolation procedures (strict hygiene , gloves, masks, etc...)


Bilateral Eye Problems
No Visible Wound
Visible Wound
Unilateral Eye Wound
Lacrimation - redness
Red Eye: Differential Diagnosis (cave: also Allergy – cage disinfectant)
Including; Conjuctivitis, Keratitis, Keratoconjunctivits
Sterile Swab for Lab Diagnostics (Bact-Virology-Antibiogram)
Therapy 6:A2 and 6:B
Virus Suspicion or Diagnosis Herpes Keratitis (or other)
Isolation if Possible
Antibiotic (+Antiviral) ointment
Improvement within 24/48hrs
No Improvement
Continue Therapy min. 1 week
Laboratory result?
Change Anitbiotics?
Add Corticosteriods?

4.7 NEUROLOGICAL CONDITIONS

W. Boardman (reviewed, S.Unwin)

These behaviour patterns are either a symptom of other well-defined diseases/disorders or related to organic brain dysfunction and/or to certain environmental conditions

Signs of abnormal or pathological behaviour

- Frequent
- Disruptive(Self injurious behaviour-SIB eg hair plucking/head butting)
- Intense

These categories are mutually exclusive

Characteristics of the pathology

- Stereotypic
  - Fine motor
    - Thumb or toe sucking
    - Eye covering
  - Whole body
    - Back flipping
    - Rocking
    - Pacing

Aetiology

- Environment/Extrinsic Determinants
- Central Nervous System/Intrinsic Determinants

Even in situations where environment is thought to play a role, other factors should be considered too eg. Most disorders are observed in individually housed animals but this has not been observed in the majority of the animals housed individually.

Prerequisites for proper Diagnosis

- Knowledge of the
  - Normal animal behaviour
  - Breeding patterns
  - Clinical history
  - Experimental history

Therapeutic strategies

- Increasing foraging devices/conditions
- Rubber toys

- Behaviour enrichment (exhibit design)
- Social integration THERE IS NO SINGLE METHOD THAT WILL RELIEVE ALL THE DISORDERS

Prevention

The development of normal behaviour in nonhuman primates is critically dependent on how a primate is reared during infancy. The optimal rearing environment is probably one in which infants are raised in species-typical social groups.
Note: Infant Husbandry is paramount

CONGENITAL CONDITIONS

Epilepsy

Is a condition of multiple and diverse etiologies that is characterized by attacks of convulsions and is sometimes associated with mental disturbances. An etiologic classification could include trauma, chemical toxicity, infectious agents and genetic predisposition.

ACQUIRED CONDITIONS

Metabolic disorders

- **Hepatic encephalopathy** Caused as a result of a deficiency in hepartic function. Insult to the liver results in increased blood ammonia due to the reduced detoxification. This manifests as neurological signs (focal defects) but can be reversed if the liver problem is corrected.

Traumatic Head Injury

- Traumatic head injury in the non-human primate is most likely to result from falls, fighting, handling mishaps or by experimental design.
- Focal brain damage includes laceration, contusions, and intra-cranial hematoma. Diffuse brain damage can take the form of diffuse axonal injury or diffuse brain swelling

TOXIC CAUSES

Uraemic syndrome

As a result of chronic renal disorder. Accumulation of metabolic by products result into clinic signs such as: wasting, itching, vomiting, poldypsia, anaemia and neurological symptoms (seizures).

DEGENERATIVE CONDITIONS

- Stroke
- Brain tumors

- Glioma
- Menningioma
- Pituitary adenoma & other metastatic tumor

Nutritional Causes

- Vitamin E deficiency- spinocerebeller degeneration & retinopathies
- Hypervitaminosis A (pyridoxine) progressive ataxia & sensory deficits

INFECTIOUS CONDITIONS

- Viral conditions
- Bacterial
- Protozoa
- Mycotic
- Parasitic

Viral infections

- Encephalomyocarditis virus
- Enteroviruses
  - Polioencephalomyelitis virus,
  - Coxsackievirus,
  - Paramyxovirus (mumps)
- Rhabdoviruses(rabies)
- Cytomegalovirus

Bacterial infections

- Tetanus
- Botulism
- Niesseria menningitidis,
- Streptococcus pneumonae
- Staphylococcus aureus
- Heamophillus influenza

Protozoal infections

- Toxoplasma gondii
- Encephalitozoan canaculi
- Trypanasoma cruzi

Mycotic infections

- Cryptococcal meningitis
- Candida albicans
- Histoplasma
- Nocadia
- These are usually diagnosed as acute viral encephalitis

Parasitic infections

- Cysticercosis
- Trichinosis

NEUROLOGICAL EMERGENCIES

- Syncope
- Seizures
- Coma
- Opiate intoxication
- CNS drug intoxication
- Stroke
- Hypertensive encephalopathy
- Acute meningitis
- Spinal cord compression
- Head trauma/spine
- Tetanus
- Botulism
- Rabies
- Viral encephalitis

4.8 DIFFERENTIAL DIAGNOSIS OF SEIZURES/COMA IN PRIMATES

W.Boardman

1. Hypoxia or ischaemia
2. Metabolic disorders
    - hypoglycaemia
    - hypocalcaemia
    - hyponatraemia or hypernatraemia
        - hypomagnesaemia
        - Vitamin B6 deficiency (pyrodoxine)
3. Drugs – barbiturates, narcotics, ketamine
4. Meningitis/Encephalitis
    - Viral
        - Enteroviruses
        - Flaviviruses
        - Measles
        - Rabies
        - Retroviruses
        - Bunyavirus
        - Arenavirus
        - Polyomavirus
        - All herpes viruses
    - Bacterial
        - Streptococcus pneumoniae
        - Neisseria meningitidis
        - Haemophilus influenzae
        - Staphylococcus aureus
        - Listeria monocytogenes
        - E coli
        - Klebsiella
        - Enterobacter
        - Proteus
        - Citrobacter
        - Pseudomonas
        - Acinetobacter
        - Salmonella
        - Shigella
        - Clostridium perfringens
    - Protozoal
    - Fungal
    - Sepsis with high fever

5. Trauma
    - Birth injury
        - Skull fracture
        - Haemorrhage
        - Cortical vein thrombosis

6. Congenital Cerebral Defects.

4.9 WOUND MANAGEMENT

W.Boardman (review S.Unwin)

AIM Establish a healthy vascular wound bed which is free of necrotic tissue, foreign debris and infection

WOUND CLASSIFICATIONS

- Clean → Created during aseptic surgery, non-traumatic, uninfected
- Clean-Contaminated → Created during aseptic surgery into the respiratory, intestinal, or urinary tracts
- Contaminated → Open, traumatic wounds with a short time lapse since trauma
- Dirty-infected → Old, traumatic wounds involving infection

CHALLENGES AND IMPEDIMENTS

- Bandaging and immobilization
- Patient mutilation
- Reduced limb mobility
- Protein deficiency
- Necrotic tissue
- Blood clots
- Dirt and debris
- Non-viable bone

PHASES OF WOUND HEALING

Inflammatory Phase
- 2-6 hr heterophils initiate phagocytosis
- 12 hr lymphocytes and monocytes
- 36 hr necrotic leukocytes accumulate

Granulation Phase
- 3-4 days in birds, 5 in mammals
- Fibroblasts synthesize collagen
- Granulation bed formed
- Epithelial cells proliferate, migrate

Granulation Phase
- Wound contraction

Maturation Phase
- Remodeling of collagen bed
- Stronger fibers re-oriented
- Takes weeks to months

WOUND ASSESSMENT

- Condition of patient
- Extent of damage
- Cause and location
- Vascular supply
- Contamination
- Age of wound

WOUND TREATMENT – THE THREE C'S

- Cleaning
- Closing
- Covering

Wound Preparation

Clip hair around the wound edges, leaving a 2-4mm margin.
Mammals: K-Y Jelly applied directly to the wound will keep hair from sticking to tissue during clipping. Rinse off when finished.

Lavage and Debridement

- Purpose: Remove debris or necrotic tissue that is present in the wound.
- Methods: Pressure lavage, wet-dry bandages, surgery.

Pressure Lavage

- Use a 20-35 cc syringe with an 18-19 gauge needle.
- Irrigation solutions: .9% Sodium Chloride, Lactated Ringers, .05% Chlorhexidine, 1% Pevodine Iodine
- Pressure generated from solution passing through syringe should dislodge most imbedded foreign debris

Wet to Dry Bandage

- Apply a gauze sponge soaked in chlorhexidine or sodium chloride directly to wound, followed by a layer of conforming gauze and vet wrap.

- As the bandage dries, foreign material is trapped in the gauze and mechanically removed with each bandage change.

Surgical Debridement

- Necrotic tissue often needs to be removed surgically to enhance wound healing.

- Requires general anesthesia or sedation therefore a veterinarian is best equipped to handle this procedure

Topical Antibiotics

- Purpose: Prevents tissue desiccation, inhibits infection, and stimulates granulation and epithelial tissue production.
- Good Products: 1% Silver Sulfadiazine (Silvadene), Bacitracin-Neomycin-Polymyxin (Neosporin), and Zinc Bacitracin.
- Avoid: Gentamicin sulfate cream, nitrofurazone, and petroleum based products

Importance of Bandage Application

- Provide support and protection to the wound
- Protect from drying and infection
- Absorb exudate and reduce swelling and hemorrhage
- Provide comfort

BANDAGE LAYERS

Primary layer

- MOST CRITICAL
- Rests directly on the wound
- Consists of adherent or non-adherent bandage material
    - Adherent material: wide-mesh gauze without cotton filler that entraps necrotic material Adherent bandages consist of wet to dry or dry to dry bandage techniques. Adherent dressings should be changed daily
    - Non-adherent bandages consist of semi-occlusive and occlusive bandage material
        - Semi-occlusive materials prevent tissue dehydration and promote epithelialization, e.g., Telfa, Tegaderm Indicated when wound is in the reparative stage of healing. Should be changed every 1-2 days
        - Occlusive materials are composed of a hydrocolloid material that enhances epithelialization. The area over the skin adheres to the bandage while the area contacting the surface of the wound absorbs fluid and creates an occlusive gel. Examples: Dermaheal. Bandages changed every 2-3 days

Secondary layer

- Allows for absorption, removal of bacteria, protection from trauma, and support
- Examples: Conforming gauze, roll cotton
- Care should be taken so that this layer is not applied too tightly such that circulation is impaired

Tertiary layer

- Holding layer. Vet wrap is the most commonly used material for this layer

- Advantages→ compliant, applies continuous pressure to wound while animal moves, doesn't remove hair or feathers

BANDAGE APPLICATION

Secondary and tertiary bandage layers should be applied such that each passing of the material overlaps the proceeding layer by 1/3 to ½. You should be able to slip at least two fingers underneath the tertiary layer. Remember, a bandage can act as a tourniquet so check it daily. Watch for signs of swelling, discoloration of the skin, or pain associated with the bandage. If these signs occur, remove the bandage until the signs resolve, then reapply

Extremities: If there are no fractures present, apply a protective dressing over the wound and have the bandage encircle the limb. A Robert Jones bandage with a splint incorporated can be applied to fractures distal to the humerus or femur. Most humeral or femoral fractures will require surgery

Head and Body: Requires more creativity in finding a way to keep the bandage on the animal. Remember, bandages can act as tourniquets so don't apply too tightly around neck or chest.

4.10 CHECKLISTS OF DISEASES & INFECTIONS REPORTED IN CHIMPANZEES

Wayne Boardman and Owen Slater

CHECKLIST OF VIRUSES OF PRIMATES WITH REPORTED INFECTIONS IN CHIMPANZEES

Virus	Reported in chimps	Not reported in chimps
Poxviridae	Monkey pox Molluscum contagiosum	Yaba virus Tanapox
Herpesviridae	Chimpanzee varicella (Varicella - zoster – Chicken pox/Shingles) Herpes hominis 1 and 11 (herpes simplex virus) Cytomegalovirus Lymphocryptovirus (Epstein Barr virus) Herpes virus pan	Herpes simiae (B virus) Simian Varicella virus Herpes virus tamarinus Herpes virus papionis – Simian agent 8 Cercopithicine herpesvirus 3, 4, 5, 8 Callitrichine herpesvirus 1 and 2 Cebine herpesvirus 1 and 2 Herpes virus ateles Herpes virus saimiri Herpes virus aotus Herpes virus saguinus Pongine herpes virus papionis Pongine herpes virus 3 (gorillas) Cercopithicine herpes virus 10, 11, 12, 13
Hepadnaviridae	Hepatitis B	
Adenvoviridae	Adenovirus	
Papovaviridae	Papillomavirus	Polyomavirus macacae – Simian Virus 40 Polyomavirus papionis – SA 12 Polyomavirus cercopitheci – Lymphotropic virus Polyomavirus hominis 1 and 2 – BK and JC virus
Parvoviridae		Simian parvovirus
Rhabdoviridae	Rabies	Vesicular stomatitis virus
Filoviridae	Ebola Cote d'Ivoire	Marburg virus Ebola reston Ebola sudan Ebola Zaire
Orthomyxoviridae Paramyxoviridae	Influenza virus A	
Paramyxovirinae	Morbillivirus - Measles (Rubella) Paramyxovirus - Parainfluenza 1, 2, 3	Paramyxovirus sanguinus
Pneumovirinae	Respiratory Syncytial Virus Human Metapneumovirus	
Togaviridae		Arterivirus - Simian haemorrhagic virus
Alphavirus		Chikungunya virus
Flaviviridae	Flavivirus -Yellow fever virus Hepatitis C like virus	Kyasanur Forest disease virus Dengue Virus GB agents

Arenaviridae		LCM –Lassa -Lymphocytic choriomeningitis Lassa virus Mopeia virus Tacaribe -Junin virus (Argentine haem v) Machupo virus (Bolivia virus)
Retroviridae	Lentivirinae SIV cpz STLV – pan – p Reported in Bonobos	T cell leukaemia type STLV Type D retroviruses SRV/D SIV agm SIV mac SIV cyn SIV stm SIV mne SIV smm SIV syk SIV mnd Gibbon Ape Leukemia virus (GaLV)
Spumavirinae	Simian Foamy virus-6 (SFV-6) SFV-7 SFV cpz	SFV-1 SFV-2 SFV-3 LK-3 SFV-8
Reoviridae	Rotavirus	Simian enterovirus
Picorniviridae	Hepatoviruses - Hepatitis A Cardiovirus - Encephalomyocarditis virus Enterovirus - Polio Coxsackie virus	Echovirus
Caliciviridae	Calici Pan paniscus type 1 Reported in Bonobos Hepatitis E	

CHECKLIST OF BACTERIAL AND MYCOTIC DISEASES REPORTED IN CHIMPANZEES

Bacterial	Mycotic
Streptococcus pneumoniae	Pneumocystis carinii
Staphylococcus aureus	Trichophyton rubrum
Corynebacteria pseudotuberculosis	Microsporum canis
Clostridium tetani	Sporothrix schenkii
Clostridium botulinum	Candida albicans
Clostridium perfringens	Cryptococcus spp
Listeria monocytogenes	Histoplasma spp
Enterobacter spp.	Geotrichum candidum
Proteus spp.	Coccidiodes immitis
Citrobacter spp.	Entomophthora coronata
Acinetobacter spp.	Nocardia spp.
Neisseria meningitidis	
E.coli	
Salmonella typhimurium/miami/oranienburg/pullorum	
Shigella	
Klebsiella pneumoniae	
Bordetella pertussis	
Pseudomonas aeruginosa	
Pseudomonas pseudomallei: Melioidosis	
Campylobacter spp.	
Mycobacterium tuberculosis/bovis	
Mycobacterium leprae	
Hemophilus influenza	
Leptospirosis	

CHECKLIST OF PARASITIC DISEASES OF CHIMPANZEES

PATHOGENIC	NON PATHOGENIC
PROTOZOA	
Giardia intestinalis (ie G.Lamblia, duodenalis)	Chilomastix spp
Entamoeba histolytica	Trichomonas spp
Trypanosoma spp	Iodamoeba buetschlii
Cyclospora cayetensis	Entamoeba chattoni
Plasmodium rodhaini	Entamoeba hartmanni
Plasmodium reichenowi	Entamoeba coli
Plasmodium schwetzi	Endolimax nana
Toxoplasmosis gondii	Blastocystis spp
Isospora belli	Troglodytella abbrassarti
Balantidium coli	
Dientamoeba fragilis (potencial)	
METAZOA	
Strongyloides fullebornii	Probstmayria spp
Strongyloides stercoralis	Physaloptera
Enterobius vermicularis	
Enterobius anthropopitheci	
Oesophagostomum stephanosomum	
Ternidens deminutus	
Ancylostoma duodenale	
Necator americanus	
Ascaris lumbricoides	
Streptopharagus sp	
Mansonella vanhoofi	
Mansonella streptocerca	
Mansonella rodhaini	
Trichuris	
Capillaria hepatica	

Schistosoma mansoni
Schistosoma haematobium
Bertiella studeri
Cystocercus
Echincoccus granulosus
Prosthenorcis elegans
Prosthenorcis spirula
Tunga penetrans
Anopluran lice
Sarcoptes scabiei
Pneumonyssus
Armillifer armillatus
Hymenolepis nana

CHECKLIST OF DISEASES OF THE DIGESTIVE SYSTEM OF CHIMPANZEES REPORTED IN THE LITERATURE

Location		Reported in chimps	Not reported in chimps but likely	Not reported in chimps
ORAL CAVITY	Dental		Dental abscesses Dental fractures Caries/Plaque/Malocclusion Periodontal disease	Necrotic stomatitis ie. Noma Oral candidiasis
	Viral oral lesions	Varicella Herpes hominis Measles		Herpes B SRV –1 SIV
	Nutritional			Scurvy Cheilosis
	Developmental			Cleft palate
	Oral hyperplasia and neoplasia	Ameloblastic odontoma		Gingival hyperplasia Squamous cell carcinoma Lymphoma Foreign body
PHARYNX, ESOPHAGUS AND STOMACH		Nasopharyngeal carcinoma Nasal polyposis Esophageal leiomyoma Trichobezoar	Vomiting	Parasitic diseases Bacterial

SMALL AND LARGE INTESTINES		Diarrhoea · Shigella Salmonella Campylobacter Measles Cytomegalovirus Rotavirus Adenovirus Intestinal Parasites Intestinal Neoplasia Appendicitis	Diarrhoea · Yersinia entercolitica/pseudotuberculosis Mycobacterium avium- intracellulare complex Mycobacterium paratuberculosis Clostridium difficile Toxic and Metabolic Diseases · PCB Amyloidoisis Protein losing enteropathy Colitis/Adenocarcinoma of Callitrichids Idiopathic colitis Constipation Idiopathic Megacolon
LIVER	Parasitic diseases	Viral diseases Yellow fever Hepatitis A, B and E	Callitrichid hepatitis
	Metabolic diseases		Fatal fatty liver syndrome
	Nutritional diseases	Amyloidosis	Cholelithiasis
	Neoplasia	Hepatocellular carcinoma associated with a. Schistosoma mansoni infection b. Myelolipoma Gall bladder adenoma	
PANCREAS		Diabetes mellitus	Adenoviral pancreatitis Parasitic disease Neoplasia

CHECKLIST OF DISEASES OF THE CARDIOVASCULAR AND LYMPHORETICULAR SYSTEMS

Encephalomyocardotis virus causing myocarditis
Atherosclerosis
Lymphangitis associated with *Enterobius spp*
Ventricular septal defect
Congestive heart failure
Hypertension
Cardiomyopathy (from Vitamin E deficiency)
Fibrosing cardiomyopathy
Aortic dissection
Atrial septal defect
Myocardial necrosis
Cardiac amyloidosis

CHECKLIST OF DISEASE OF THE RESPIRATORY SYSTEM IN CHIMPANZEES

Air sacculitis
Tracheo bronchial foreign body
Asthma
Rhinitis
Diaphragmatic hernia
RSV infection
HMPV infection
Parainfluenza 1, 2 and 3
Measles
Influenza A
Adenovirus
Tuberculosis
Streptococcus pneumoniae
Kelbsiella pneumoniae
Bordetella pertussis
Pneumocystis carinii
Pneumonyssus spp
Toxoplasmosis
Enterobiasis

CHECKLIST OF DISEASES OF THE UROGENITAL SYSTEM

System		Reported in Chimps	Not reported in Chimps
URINARY SYSTEM	Renal Disease	Suppurative nephritis and pyelonephritis Renal carcinoma	Glomerulonephritis Interstitial nephritis Parasitic nephritis Nephrosis Renal amyloidosis
	Urolithiasis and Renal Calcification		Urinary calculi Nephrocalcinosis Cystitis Congenital malformations Neoplastic disease
GENITAL SYSTEM **FEMALE**	Vulva and Vagina	Mycoplasmosis infection Herpes hominis 2 infection (Pygmy Chimps)	Parasitic infection Neoplasia
	Uterine Cervix		Cervicitis Cervical polyps
	Uterine body	Leiomyoma of the myometrium Adenomyosis	Endometritis Endometrial hyperplasia Endometrial polyps Adenomyosis
	Oviduct, Broad Ligament and Ovary		Salpingitis Parovarian Cysts Follicular cysts Luteal cysts
	Neoplasia	Sertoli-Leydig tumour	Benign Ovarian teratoma Dysgerminoma Ovarian carcinoma
	Mammary Gland		Mastitis Mammary neoplasia Endometriosis

	Disorders of Menstruation	Dysmenorrhoea	Amenorrheoa Abnormal uterine bleeding
	Obstetrical Problems Placental disorders	Chorioamnionitis	Amniotic band syndrome Extrachorial placenta Retained placenta
	Pregnancy Loss		**Gram pos bacteria** *Listeria, Streptococcus* **Gram neg bacteria** *E.coli, Yersinia pseudotuberculosis, Salmonella heidelberg, Shigella flexneri* *Mycoplasmas* *Leptospira copenhageni* **Viral** Measles, Rubella, Mumps
	Complications of Pregnancy		Ectopic pregnancy Mummified fetus Placenta previa Placenta abruptio Ruptured uterus Hypertension Preeclampsia/Eclampsia Pregnancy induced Glucose intolerance Coagulation disorders Hydronephrosis of pregnancy
	Maternal-Fetal Interactions	Dystocia Uterine fibroid Breech	Pre term labour Aspiration distress Umbilical cord complications Erythroblastosis foetalis
MALE		Sertoli-Leydig cell tumors Testicular interstitial cell tumors Urethral obstruction	Disorder of the Penis, Prepuce, Testicles, Scrotum & Glands

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4.11. HUMAN INFECTIONS DISEASES THAT HAVE CAUSED INFECTION AND DISEASE IN APES

W. Boardman. Review: S.Unwin

A good general reference on Zoonotic disease is Zoonoses: Infectious disease transmissible from animals to humans, Krauss et al (eds) 3rd ed. ASM press 2003. Management strategies of Zoonotic diseases can be based on disease contingency planning principles (section 3.2.)

HUMAN BACTERIA THAT HAVE CAUSED INFECTION AND DISEASE IN APES

Pathogen	Pathology/Symptoms in Apes	Species Affected	Pathology/Symptoms in Humans	Route of Transmission	Survival	References
Campylobacter jejuni	Enterocolitis	ALL	Enteritis, colitis	Faecal/oral	UNK	Ott-Joslin 83 & 93
Bordetella pertussis		Chimps	Whooping cough	aerosol		Kalter 80
E.coli		Chimp	Gastroenteritis	Faecal/oral		Benirschke et al 80 Van Kruinigen etal 91
Leptospirosis		Chimp	Renal and hepatic disease	Faecal/oral		Benirschke et al 80
M.tuberculosis	Same as humans	All	Pulmonary and extra pulmonary disease	aerosol		Ott-Joslin 83 & 93
Mycoplasma						
Salmonella	Enterocolitis	All	Typhoid fever	Faecal/oral	2 h @75% humidity, 24^{o}C	Ott Joslin 83 & 93 McDermid et al 96
Shigella		Gorilla				
Treponema pertenue	Same as humans	Gorilla	Lesions of skin, bones and mucosa	contact		Benirschke et al 80
Yersinia enterocolitica	Diarrhoea	All	Diarrhoea	Faecal/oral		Swenson
Streptococcus pneumoniae	Respiratory infection (often carriers), meningitis	Chimp	Causes pneumococcal meningitis. High prevalence of asymptomatic carriers	Aerosol	Ubiquitous	Kondgen 08, Unwin (unpub.)

HUMAN VIRUSES THAT HAVE CAUSED INFECTION AND DISEASE IN APES

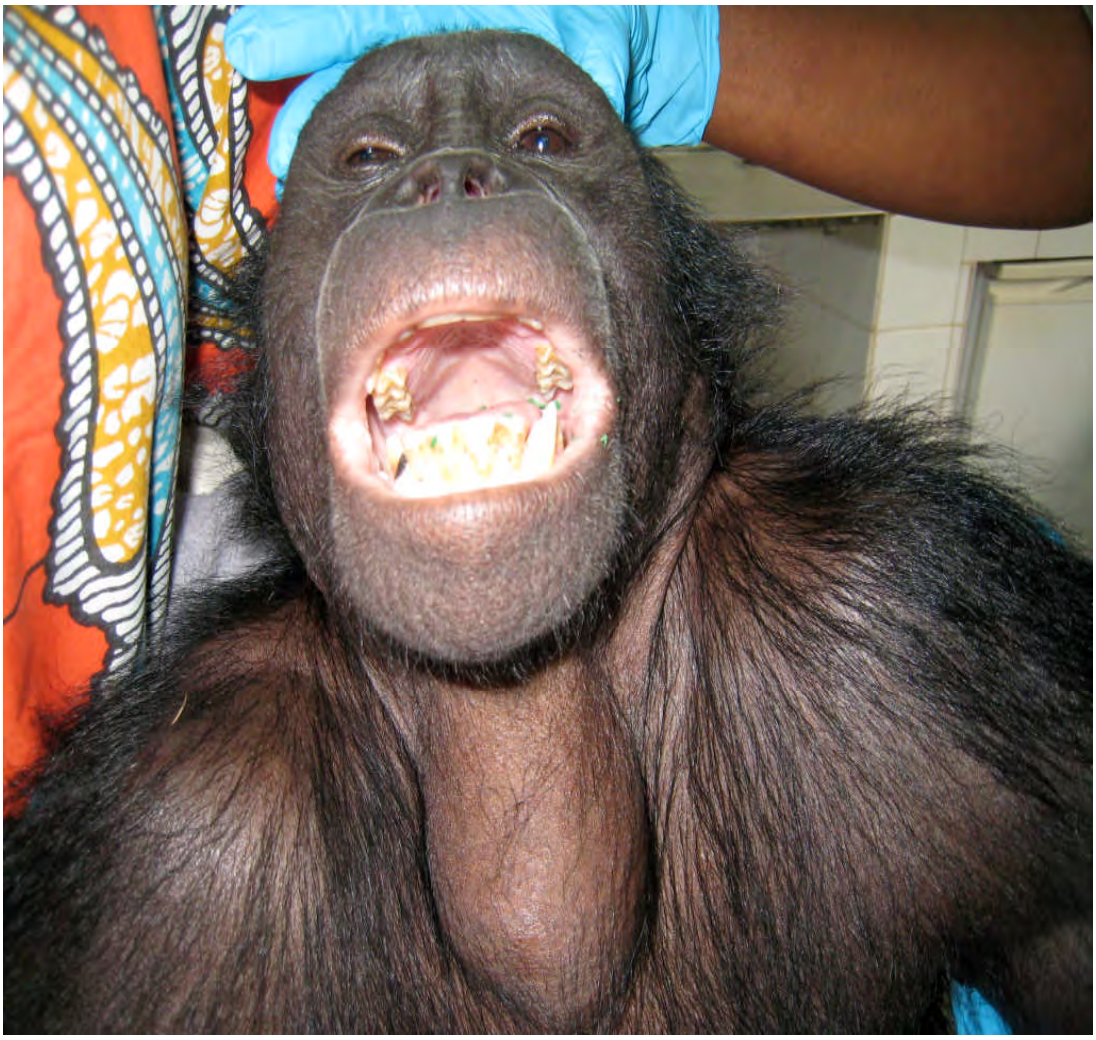
Pathogen	Pathology/Symptoms in Apes	Species Affected	Pathology/Symptoms in Humans	Route of Transmission	Survival	References
Adenovirus	Respiratory and gastrointestinal -often asymptomatic	Chimps	Respiratory and gastrointestinal – often asymptomatic	Aerosol and Contact	Resists temps of 60°C	Dick et al 64
Arbovirus Yellow fever	Haemorrhagic fever	Chimps Gorillas	Haemorrhagic fever	mosquito	**150-17O days in soil** 15 days in food	Kalter 80
Coxsackie	Fatal respiratory and enteric syndrome	Chimp	Often asymptomatic or wide variety of problems			Kapikian 96 Ott-Joslin 83 & 93
Hepatitis A	Asymptomatic	All	Acute hepatitis	Faecal/oral	Several weeks @ 25°C	Anan'ev et al 84 Bielitzki 96
Hepatitis B	Often asymptomatic	All	Acute and chronic disease, predisposes to liver cancer	Blood/Sexual	At least 7 days on fomites	Linneman et al 84 Ott Joslin 83 & 93 Warren et al 98 Zuckerman et al 78
Hepatitis C	Asymptomatic, possible chronic hepatitis, cirrhosis	Chimp	Usually asymptomatic, often followed by chronic disease	Blood		
Herpes hominis 1 & 2	Oral, lingual and genital blisters and ulcers, fever, conjunctivitis, diarrhoea, CNS signs	All	Oral, lingual and genital blisters and ulcers, fever, conjunctivitis, diarrhoea, CNS signs	Aerosol Contact sexual	Hours on fomites Days on dry absorbent surface	Ablashi et al 79 Benirschke et al 80 Eberle et al 89 Helsdstab et al 81 Neubauer et al 79
Herpes zoster	Chicken pox and Shingles	All	Chicken pox and Shingles			Ott Joslin 83 & 93 Padovan et al 86 Rabin et al 80
Epstein Barr	Similar to humans	Chimp Gorilla	Glandular fever			Warren at al 98
HTLV –1	Lymphoma	Gorilla	Lymphoma	Sexual Blood		Srivastava et al 86 Lee et al 85
Influenza	Influenza signs but worse than in humans	Chimp Gibbon	Influenza signs	Aerosol	Less than 3 days food **1-2 days on hard surface** Less than 12h	Kalter 80 Ott Joslin 83 & 93 Johnsen et al 71

					porous surface Up to 4m at 2^0C	
Measles	Asymptomatic to fatal – often with Fever, rash, conjunctivitis, GI and respiratory signs	All	Fever, rash, conjunctivitis, GI and respiratory signs	Aerosol Contact	Only 2 hours infectivity from droplets	Ott Joslin 83 & 93 Hastings et al 91
Mumps	Parotiditis and orchitis	All	Parotiditis and orchitis			
Parainfluenza type 3	Flu like signs	Chimp, Gorilla Gibbon	Few signs.	Aerosol	4-10 hours on absorptive surfaces	Clements et al 91 Jones et al 84
Papilloma virus	Oral hyperplasia	Chimps	Common and genital warts	Contact Blood	Resistant to disinfectants	Bielitzki 96
Polio	Meningitis, encephalomyelitis and paralysis	All	Meningitis, encephalomyelitis and paralysis	Faecal/Oral	150-170 days in soil 50 days in cockroaches 15 days in food	Ott Joslin 83 & 93 Froeschle et al 65
Poxvirus	Molluscum contagiosum; benign small skin nodules	Chimps	Molluscum contagiosum; benign small skin nodules	Contact		Ott Joslin 83 & 93
Respiratory Syncytial Virus	Respiratory Infections incl Bronchopneumonia	Chimps	Respiratory Infections incl Bronchopneumonia	Aerosol	1-6 hours on fomites	Clarke et al 94 Lerche et al 93 Richardson et al 81
Rhinovirus	Subclinical to mild URT signs	Chimp Gibbon	Common cold, rhinitis	Aerosol Faecal/Oral		Ott Joslin 83 & 93 Dick et al 68
Rotavirus	Gastroenteritis i.e. diarrhoea and vomiting	Chimp Gorilla Orang	Gastroenteritis i.e. diarrhoea and vomiting	Faecal/Oral	Resist to disinfectants Aerosols stable @ 50% humidity for 24h	Ott Joslin 83 & 93 Ashley et al 78 Ijaz et al 94
Human Metapneumovirus	Respiratory infections	Chimp, mandrill	Respiratory infections – often found in association with bacterial infections	Aerosol	Very common in humans (carriers). Aerosol spread. Human to wild chimp infection confirmed	Kondgen et al 08

HUMAN PARASITES THAT HAVE CAUSED INFECTION AND DISEASE IN APES

Pathogen	Pathology/Symptoms in Apes	Species Affected	Pathology/Symptoms in Humans	Route of Transmission	Survival	References
Ancylostoma duodenale	Same as humans	Chimps Gorillas	Hookworm disease – anaemia, intestinal and pulmonary signs	Faecal-oral		Benirschke et al 80
Ascaris lumbricoides	Diarrhoea	Chimps Gorillas	Diarrhoea	Faecal-oral		Benirschke et al 80
Balantidium coli	Same as humans	Chimps Gorillas	Diarrhoea and dysentery	Faecal-oral		Marsden et al 91 Lee et al 90 Teare et al 82
Cryptosporidium	Same as humans					
Echinococcus mulitlocularis	Often asymptomatic	Gorilla	Hydatid disease	Faecal-oral		Benirschke et al 80 Kondo et al 96
Entamoeba histolytica	Amoebic dysentery	All	Amoebic dysentery	Faecal-oral		
Enterobius vermicularis	Can be fatal in chimps	All	Anal itch	Faecal-oral		Ott Joslin 83 & 93
Giardia lamblia	Diarrhoea	All	Diarrhoea	Faecal-oral		Benirschke et al 80
Isospora belli	Diarrhoea	All	Diarrhoea fever	Faecal-oral		Warren at al 98
Loa loa Mansonella	Same as humans	Gorilla	Filariasis	Insect vectors		Bain et al 95 Haberman et al 68
Sarcoptes scabei	Same as humans	All	Scabies	Contact		Kalema et al 98 Macfie 96
Schistosoma sp	Sam as human	All	Bilharzia - Urinary and intestinal pathology	transdermal		Kalter 80
Strongyloides stercoralis	enterocolitis	All	Intestinal and pulmonary disease	Faecal-oral		Benirschke et al 80 Ashford et al 90
Trichuris	Often asymptomatic	All	Often asymptomatic	Faecal-oral	Resistant to disinfect ants	Benirschke et al 80

SECTION 5
CURRENT THERAPY



SECTION 5 – CURRENT THERAPY

5.1 TRADE IN GREAT APES AND WILDLIFE LAW ENFORCEMENT – Challenges and Solutions
A Case Study of Wildlife in Cameroon

Ofir Dori
The Last Great Ape organisation (LAGA). LAGA has been tasked by the Cameroonian Government to head up their Wildlife Law Enforcement units.

EXAMPLE: 'The Taiping Four Gorillas':

- Originated in Cameroon
- Through Ibadan Nigeria
- To the Taiping zoo in Malaysia

What Price gorillas? $US1.6 million !

Figure 1: the Taiping Four gorillas at there new 'home' in Pretoria Zoo.

Information from the Nigerian Federal Investigation Committee:

- Originated in Cameroon
- Through Ibadan Nigeria
- To the Taiping zoo in Malaysia

(vii) Karriah Mohd. Sharif, the Director of Malaysia Wildlife Dept. cleared the country from the scandal, but acknowledged the possibility that the gorilla could have been illegally obtained in Nigeria.

(viii) The gorillas were offered to the Taiping Zoo for the price of $1.6 million by the seller.

(ix) International postcard campaign to Cameroon, Nigeria and Malaysia on the

The animals were shipped back to Pretoria Zoo in South Africa (Figure 1), and finally, 4 ½ years after being taken from Cameroon, they were returned, to the Limbe Wildlife Centre, in 2008. This was after repeated official requests from the Cameroonian Government.

Overview

There is a huge financial incentive drives the international trade in great apes. Zoos can play a negative role in the illegal trade in apes through uncontrolled use of dealers. Trade in great apes is international and regulated under the CITES (Convention on Illegal trade in Endangered Species). Laws to regulate commercial trade in threatened species do exist in the sub-region but they are barely enforced in the field – across Africa. Most of the countries in West and Central Africa still on zero wildlife prosecutions. There is a real need for developing effective Wildlife Law Enforcement (Figure 2 and 3).

Figure 2 and 3. there is a real need for developing effective Wildlife Law Enforcement

Is there real trade in great apes ?

EXAMPLE: Cameroon Apes and Drugs

January 2006- an operation involving drugs and apes trade in Cameroon was being investigated.
Four large sacks of marijuana, weighing about 50Kg were seized with a young chimpanzee. Another kind of drug was also caught in possession of the dealer which he admitted was cocaine (Figures 4, 5 and 6). The drugs and wildlife dealer had been employing at least 4 poachers and had been trading other protected primates regularly.

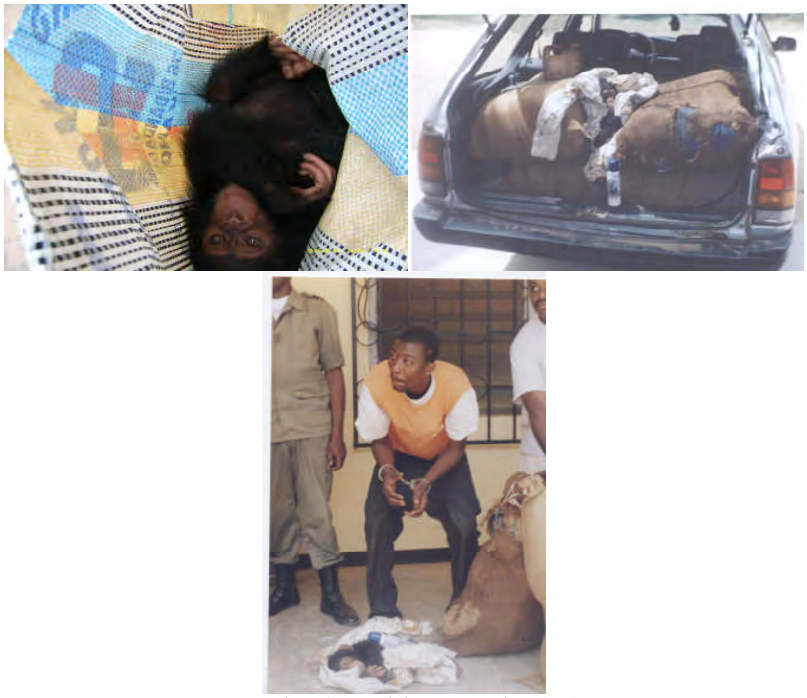


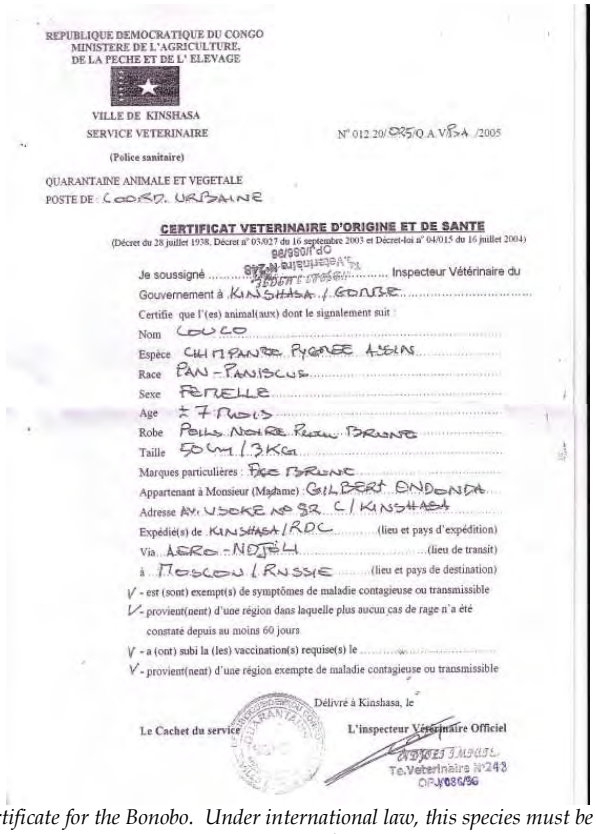
Figure 4, 5 and 6. Ape and drug smuggling in Cameroon

EXAMPLE: DRC – Bonobo Smuggling Affair

On December 2005, two wildlife dealers, a Ukrainian and a Congolese with a Russian Passport, boarded an Air France flight from Kinshasa with a final destination Russia. In a hand bag inside the plane they transported a baby Bonobo. This was well known to Air France as the two presented a permit from the Congolese Ministry of Agriculture - Certificate of veterinary origin and health, figure 7, and were allowed to take the animal on board. Their passports show they had flown frequently on the Moscow-Kinshasa route suggesting this was not their first time smuggling.

In France, following an intervention by an activist, custom authorities in the Roissy airport held the subjects with the Bonobo. The Bonobo had been confiscated but the subjects released and allowed to continue their journey to Russia. The airport authorities intended to euthanase the bonobo due to Ebola fear.

This shows that it is not only African countries that are lagging behind in great ape enforcement. Foreign nationals are involved and they can move their base from one African country to another.



REPUBLIQUE DEMOCRATIQUE DU CONGO
MINISTERE DE L'AGRICULTURE,
DE LA PECHE ET DE L' ELEVAGE

VILLE DE KINSHASA
SERVICE VETERINAIRE
(Police sanitaire)

N° 012.20/025/Q.A.V/BSA /2005

QUARANTAINE ANIMALE ET VEGETALE
POSTE DE : LODJA URBAINE

CERTIFICAT VETERINAIRE D'ORIGINE ET DE SANTE
(Décret du 28 juillet 1938, Décret n° 03/027 du 16 septembre 2003 et Décret-loi n° 04/015 du 16 juillet 2004)

Je soussigné ..... Inspecteur Vétérinaire du
Gouvernement à KINSHASA / GOMBE
Certifie que l'(es) animal(aux) dont le signalement suit :
Nom LOUCO
Espèce CHIMPANZE PYGMEE ALBIN
Race PAN - PANISCUS
Sexe FEMELLE
Age ± 7 MOIS
Robe POILS NOIRS PEAU BRUNE
Taille 50 CM / 3 KG
Marques particulières : FACE BRUNE
Appartenant à Monsieur (Madame) : GILBERT ENDONDA
Adresse AV. USOKE N° 32 C/ KINSHASA
Expédié(s) de KINSHASA / RDC (lieu et pays d'expédition)
Via AERO - NDJILI (lieu de transit)
à MOSCOU / RUSSIE (lieu et pays de destination)
V - est (sont) exempt(s) de symptômes de maladie contagieuse ou transmissible
V - provient(nent) d'une région dans laquelle plus aucun cas de rage n'a été constaté depuis au moins 60 jours
V - a (ont) subi la (les) vaccination(s) requise(s) le .....
V - provient(nent) d'une région exempte de maladie contagieuse ou transmissible

Délivré à Kinshasa, le

Le Cachet du service

L'inspecteur Vétérinaire Officiel

Te.Veterinaire N°243
OPJ/086/06

Figure 7. Veterinary Certificate for the Bonobo. Under international law, this species must be transported with CITES paperwork.

EXAMPLE: Republic of Congo – September 06- National Ape Dealer.

The authorities have arrested a trafficker who was selling wildlife just in the center of Brazzaville in particular chimps. Apparently, he had already sold many animals including 5 baby gorillas and 10 chimpanzees. After three days in prison, he was released after an unknown person asked to release the trafficker.

National dealers can be specialized

Dealers can be "Protected"

EXAMPLE - Internet – covering many countries – E.G Michelle Gardener – South Africa

LA REPUBLIQUE DU CAMEROUN
PAIX-TRAVAIL-PATRIE

THE REPUBLIC OF CAMEROON
PEACE-WORK-FATHERLAND

EXPORTATION LICENSE ON PETS AND LIVESTOCK

REFRENCE 4135

...is license is issued to MRS MICHELLE GARDNER
The ministry of fishery, livestock and Animal husbandry in collaboration with custom officials has permitted this pet raising company in Cameroon to ship out of the country. This is enforced with **Art No 2/30/31/1987** declaration that breeders can export ~~apes~~ to different countries. This permit is valid until 2010.

This permit is restricted only to AFRICA, EUROPE, U.S.A, CANADA, AND ASIA. Any thing out of these countries is out of reference No 2/30/31/1987

Category License

Birds/Eggs ✓
Dogs ✓
Monkeys ✓

Figure 8. Open licence for Michelle gardener to transport 'pets and livestock'

Internet connects supply with demand and offers more opportunities for an illegal deal. Internet wildlife fraud is on the rise.

ORGANISED CRIME?

Figure 9. Overview of wildlife trafficking routs out of Cameroon, and highlighting where enforcement operations have taken place to date.

Professional organized trade operates beside opportunistic speculators trying to join the game. Often in enforcement you meet the "losers" of the trade

Figure 10 from $US100 to

Figure 11 $US100 000?

Why is the law not applied ?

Corruption, corruption, corruption

- Lack of measurable standards.
- Lack of communication between governmental agencies.
- Lack of information to the public

Figure 12. Can Wildlife Law Enforcement Work?

LAGA's collaboration with the Government of Cameroon

INVESTIGATIONS

Investigators, undercover agents and informers gather precise information so that dealers in meat and the products of threatened species can be arrested in the act, producing concrete evidence for the courts.

OPERATIONS

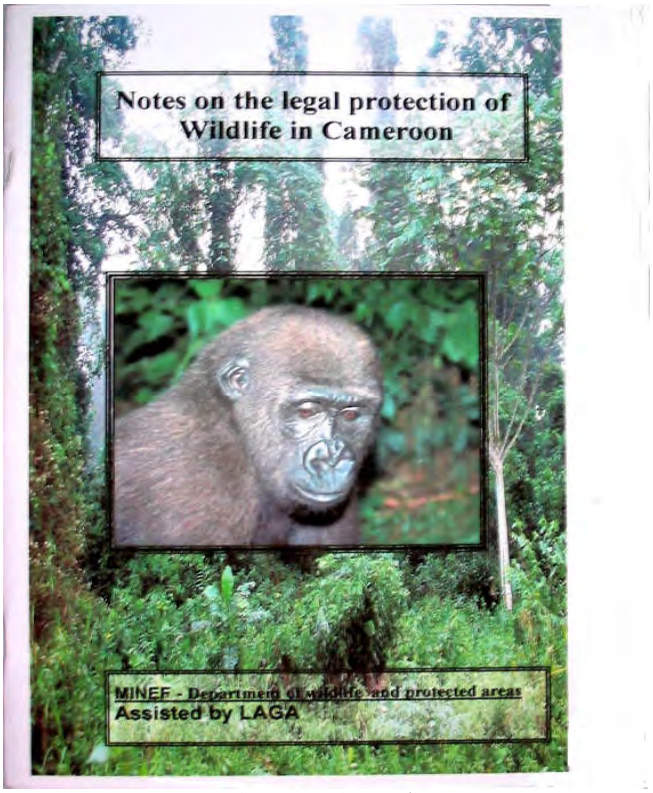
LAGA technically assists MINFOF and the forces of law and order to arrest violators and to channel complaint reports to the courts. LAGA closely supervises operations in the field. Around 83% of the operations are associated with documented bribing attempts.

Fighting corruption.

Figure 13. LAGA not only deals with illegal primate trade

LEGAL ASSISTANCE

LAGA formed a legal team to assist in the administrative procedures of prosecuting the first wildlife cases known in the courts of Cameroon. They have similar figures for documented bribing attempts in the courts.



Figre 14. Using Media

MEDIA

Newsflashes on national TV and radio news. Written press concerning the success of the operations and positive court rulings. Education of the public on the change in enforcement , highlighting the increased deterrent, and classification of the illegal wildlife trade as a criminal activity.

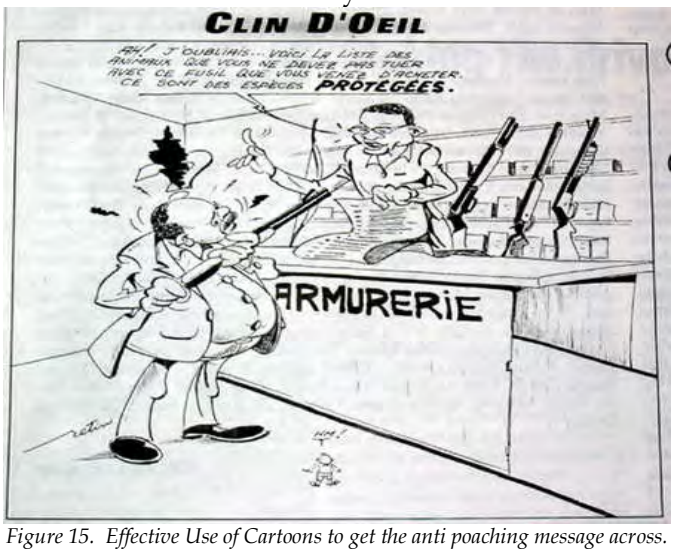


Figure 15. Effective Use of Cartoons to get the anti poaching message across.

Analysis of Solutions

- One measurable standard – Prosecutions – see below.

- Follow-up of the entire process across its different stages.
- Tight supervision in the field.
- Strong anti-corruption policy.

No	Case name	Offence	Profile	Remarks	Status
1	Bityeki	dealing with live chimp +drug	pet trade	Drug dealer arrested with 50 kg Marijuana, activates 5 poachers	locked while on trial
2	Markova Yuliya	dealing with a bonobo	pet trade	DRC, international ukranian ape dealer	fugitive under chase
3	Ondonda Zhilber	dealing with bonobo	pet trade	DRC, international ape dealer, Russian passport	fugitive under chase
4	Eyong James	dealing with elephants products + murder	Ivory dealers	Killed 270 elephants, accomplice of murder of an ecoguard	locked while on trial
5	camara Mohamed	dealing with ivory	Ivory dealers	ivoirian connection to ivory trade	locked while on trial
6	Keita Nounke	dealing with ivory	Ivory dealers	Guinnean connection to ivory trade	locked while on trial
7	Sanoh Ibrahim	dealing with ivory	Ivory dealers	Guinnean connection to ivory trade	locked while on trial
8	Emene Wilfried	Selling young chimpanzee	pet trade	ape dealer trafikking between East and Yaounde	locked while on trial
9	Ahanda Abomo	detention of a live drill	Pet trade	negligence of NP project	locked while on trial
10	Eyanga	detention of a live drill	Pet trade	negligence of NP project	free while on trial
11	Angoulla Angoulla	Trade in crocodiles	bushmeat dealer	worker of logging company	locked while on trial
12	Sanoh Ibrahim	dealing with ivory	ivory dealers	repeated offender	locked while on trial
13	Adamou babouga	dealing with ivory	Ivory dealers	bamenda	locked while on trial
14	Njung Bonshe	dealing with chimpanzee mea	bushmeat dealer	bamenda	locked while on trial
15	Jikombe Daouda	dealing with ivory	ivory dealers	Wealthy High personality spends half time in France	locked while on trial
16	Goumbe Inoussa	dealing with ivory	ivory dealers	Foumban	locked while on trial
17	Makan Samuel	dealing with ivory	ivory dealers	repeated offender, arrest on a ship, international trade	locked while on trial
18	souleyman ali	dealing with lion	Lion trade	Laundring illegal trade in the cover of a hunting safari	locked while on trial
19	tonye Nicolas	selling live chimpanzee	pet trade	Edéa	fugitive under chase
20	boulong	selling live chimpanzee	pet trade	Edea	fugitive under chase
21	Cathérine fouda	Protected animals	International dealer in protected animals	Underground large shop, international trafick, Daughter of first mayor of Yaounde, sister to a minister	free while on trial
22	Teng Tonny	dealing with ivory	Ivory dealers	Asian, 3.9 tonnes affair	fugitive under chase
23	Teng Andrew	dealing with ivory	Ivory dealers	Asian, 3.9 tonnes affair	fugitive under chase
24	Teng Nora	dealing with ivory	Ivory dealers	Asian, 3.9 tonnes affair	fugitive under chase

SUMMARY

- **Extensive and lucrative trade in great apes exists**
- **Shelters contact point with the trade is specific**
- **Wildlife Law Enforcement is possible – build expectations**

5.2 PLACENTAL RETENTION IN A BONOBO (*Pan paniscus*)

Michel Halbwax (Max-Planck Institute for Evolutionary Anthropology), Anne-Marie Ngalula (Lola ya bonobo) and Crispin Mahamba (Lola ya bonobo)

See the full article at: J Med Primatol. 2009 V38(3):171-4.

Overview of female bonobo reproduction

- Sexual maturity in captivity between 6 and 11 years of age
- First offspring: between 13 and 14 years old
- Interbirth interval ~ 4 to 6 years
- Gestational length: between 220 and 230 days

Pre-parturition history

- 11 years old female (Kalina), 29Kg
- Primiparous
- No contraception history
- Last estrus observed: 210 days before birth date
- Isolated from her social group 2 months before the presumed birth date.

Placental retention

- Normally:
  placenta is delivered within ½ hour after birth
  with a mean of 20 minutes *(Lola Ya Bonobo sanctuary)*

- In this case:

11 Hours postpartum (HO+11): no expulsion despite regular contractions of the uterus (figure 1)

Treatment: medication

H0+11: Injection IM of 5 IU of oxytocine (Syntocinon® 5 IU/1 ml)
additional uterine contractions were observed but no expulsion

Figure 1. Placenta retained 11 hours after bith, despite regular contractions.

H0+27: umbilical cord broke
half of the placenta expelled
injection IM of 10 IU of oxytocine (Syntocinon® 5 IU/1 ml)
unsuccessful in expelling the remainder of the placenta

H0+27.5: 10 IU more of oxytocine (Syntocinon® 5 IU/1 ml), IM
no amelioration

H0+28: anesthesia and intervention decided
Anesthesia by dart: 1 ml Ketamine IM (Ketamine 1000®, 100mg/ml) 1 ml medetomidine IM (Domitor®, 1mg/ml)

Supportive treatment during the anesthesia
350 ml NaCl 0.9%, IV
10 ml hypertonic glucose (50%), IV
400 mg of amoxycillin (Clamoxyl® 1g/5ml), IV
75 mg of diclofenac (Voltarene® 75 mg/3ml), IM

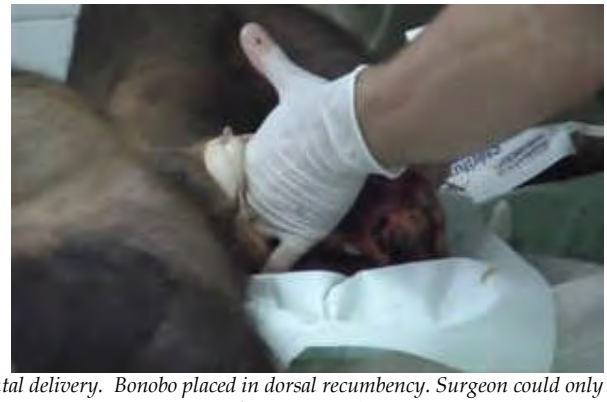
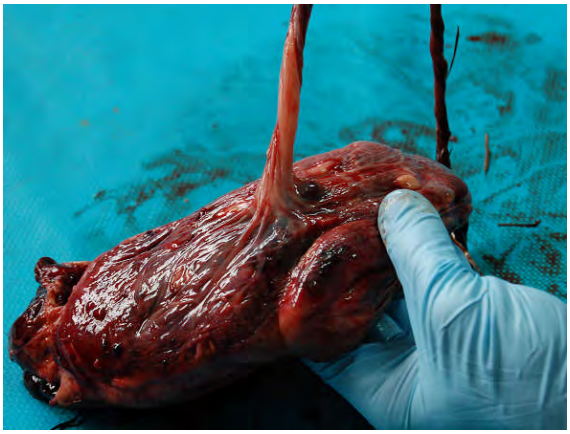


Figure 2 and 3 – placental delivery. Bonobo placed in dorsal recumbency. Surgeon could only get 7cm of his hand into the tract to assist.

Figure 4. Force out the uterus' contents with belly massage…

Figure 5….and placenta turned in clockwise and anticlockwise direction…

Figure 6 and 7…to deliver the whole placenta



Medication after the delivery:
0.2 mg IM of methylergometrine (methergin® 0.2 mg/ml)
5 mg IM of atipamezole hydrochloride (Antisedan® 5 mg/ml)

Probable scenario?

1: Strict placental retention with no bleeding: the placenta remained fixed to the uterus by cotyledons despite the contractions

2: Over the next 11-27 hours postpartum, the placenta was partially expelled while the cervix also began to close

3: placenta trapped in the uterus by the closed cervix. This would cause the placenta to develop an hourglass shape.

The following days:
D0+2: fever (38.1° C), diarrhea, muco-hemorrhagic-non smelly lochia

Additional medication:
40 mg/kg/*per os* b.i.d of metronidazole (flagyl® 125 mg/5ml)

The dam's health status improved rapidly over the following days. The infant remained healthy.

Medication summary:
Amoxycillin: 9 days from the first day postpartum
Diclofenac: 2 days from the first day postpartum
Metronidazole: 7 days from the second day postpartum
Probiotics, rice..: 5 days from the second day postpartum
Methylergometrine: once 1 day postpartum
Iron: started 6 months into the pregnancy and administrated for 4 more days postpartum.

Figure 8. Recovered mother, with baby.

5.3 REINTRODUCTION DE GORILLES ET DE CHIMPANZES DANS LEUR MILIEU NATUREL: Bientot 10 Annees D'experience De Trois Projets Differents

S. Mahe (Vet, PPG Gabon)

Introduction

- Espèces menacées par
- Déforestation
- Braconnage
- Maladies
- L'avenir pour les orphelins
- Sanctuaires
- Programme de réintroduction

- Un projet de réintroduction, selon les recommandations de l'UICN, doit avoir pour but principal de rétablir une population viable et autonome dans le milieu sauvage.

HELP CONGO	PPG Congo	PPG Gabon
Renforcement	**Réintroduction**	
Chimpanzés (*Pan troglodytes troglodytes*)	**Gorilles *(Gorilla gorilla gorilla)***	
Congo Brazzaville Parc National de Conkouati Douli	**Congo Brazzaville Réserve de Lesio Louna et de Léfini**	**Gabon Parc National des plateaux Batékés**

	HELP	PPG Congo	PPG Gabon
Nombre d'individus au total	**43 Chimpanzés 32 relâchés 11 sur les îles**	**21 Gorilles 17 relâchés 4 en cage**	**23 Gorilles Tous relâchés**
Origine des individus	**Congo**	**Congo**	**Gabon, et UK (zoo de Howletts)**
Répartition des relâchés	**Mode fusion fission Sex ratio : 7.25 âge 3 à 19 ans**	**Groupe 1: sex ratio 2.3 âge 12 à 17 ans Groupe2: sex ratio 4.5 âge 6 à 9,5 ans Groupe 3 : sex ratio 0.3 âge 3 à 6 ans**	**Groupe 1: sex ratio 6.8 âge de 5 à 9,5 ans Groupe 2: sex ratio 4.5 âge de 3 à 6,5 ans**

	HELP	PPG Congo	PPG Gabon
Personnel	**20 salariés congolais 2 expatriés indemnisés, nombreux bénévoles**	**32 salariés congolais 2 expatriés salariés**	**5 salariés gabonais 4 expatriés salariés**
Financements	**Recherche de financements auprès des associations, des entreprises, des zoos**	**Fondation John Aspinall**	

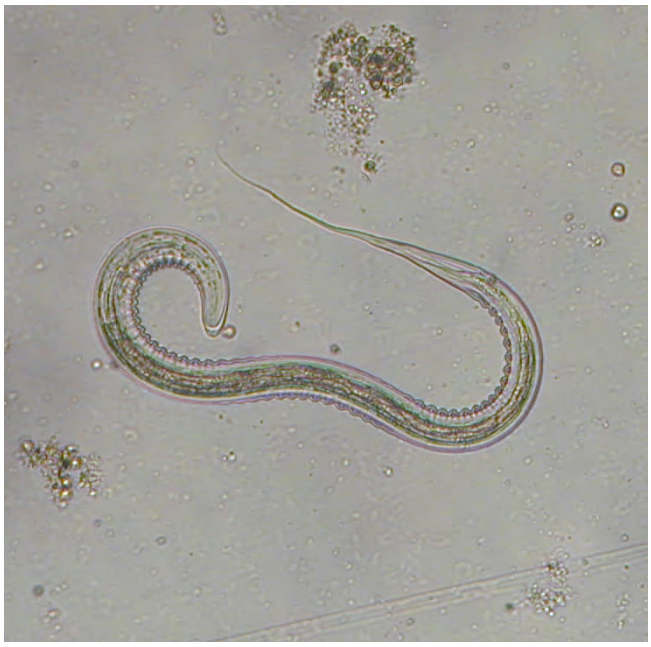
Préparation au relâcher: choix du site

- Choix du site après expertise et en accord avec les populations locales
- Nourriture adaptée et suffisante
- Présence ou non de congénères
- Éloignement des habitations

HELP	PPG	PPG
Site du triangle : forêt primaire, secondaire et inondable, 22 km2 délimité par des rivières franchissables	Réserve de Lésio Louna: forêt galerie délimité par des barrières naturelles (rivières, falaises, savanes)	Forets galeries au sein du Sanctuaire de la Mpassa, délimitées par rivières et savanes
Densité chimpanzés sauvages 0,17-0,33 chimpanzé/km2	Réintroduction au sein de l'ancienne aire de répartition de l'espèce	

Protocole sanitaire

- Il existe un risque sanitaire pour les populations sauvages
- Un protocole sanitaire est mis en place selon les recommandations de l'UICN
- Pour les primates non humains
- Pour les humains



- Quarantaine
- Tuberculination paupière (une seule)
- Examen parasitologique des selles
- Virologie: HIV/SIV,HTLV/STLV, Hep A et B
- Hématologie, biochimie
- Vaccination: DTpolio, ROR (gorilles)
- Vermifugation préventive avant le relâcher

Stratégie de relâcher douce

HELP

Apprentissage sur les îles: recherche de nourriture, construction de nids, établissements de liens sociaux

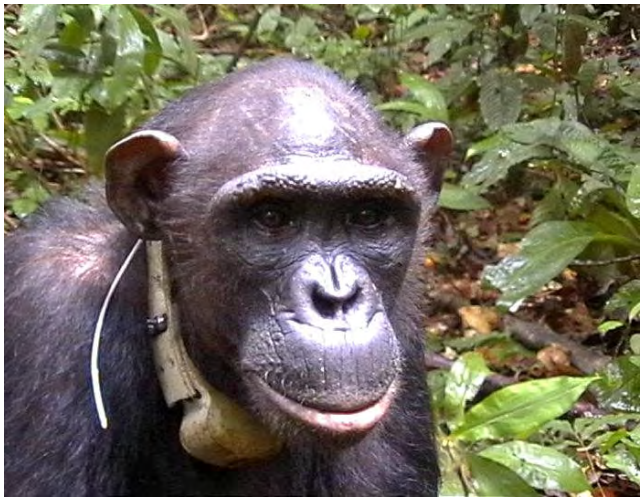
Relâcher dans un site différents, individus regroupés par affinité

PPG

Apprentissage et relâcher sur le même site: cage et alimentation en forêt sont progressivement supprimés

Suivi des individus intensif

HELP



Télémétrie, colliers émetteurs à changer tous les ans, sur tous les individus la première année, puis sur certains seulement

PPG

Accompagnement des animaux en foret quand ils sont en cage puis pistage, Colliers impossibles

Méthode de suivi

- Suivi des individus toute la journée la première années
- Localisation au moins quotidienne pour les gorilles, mais arrêt du suivi direct à partir de l'âge adulte
- Suivi de plus en plus espacé pour les chimpanzés
- Scan sampling régulier pour les chimpanzés, au début seulement pour les gorilles

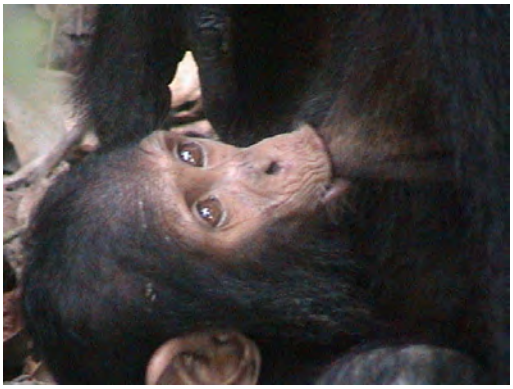
Résultats- taux de survie

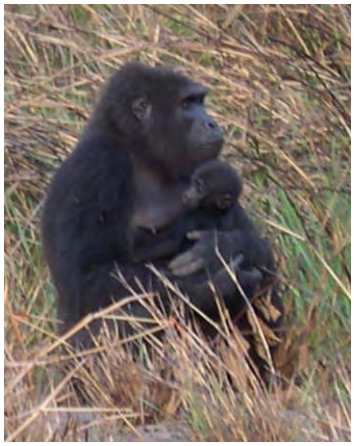
	HELP	PPG Congo	PPG Gabon
Individus relâchés	38	27	29
Suivis	22	17	23
Remis en cage		4	
Non observés	10	1	1
Décédés	6	5	5
Taux de survie	57,9 – 84,2 %	77,8 – 81,5 %	79,3 – 82,8 %

Causes des décès

HELP	PPG Congo	PPG Gabon
1 oesophagostome 1 noyade 4 agressions chimpanzés	1 cause inconnue 1 étranglé collier 3 agressions gorilles	Gorilles gabonais :1 causes inconnue, 1 noyade, Gorilles d'UK : 1 appendicite, 1oesophagostome, 1 péritonite cause inconnue

Naissances





HELP	PPG Congo	PPG Gabon
7 naissances en foret, 5 décédés, 2 en bonne santé (mâle 2,5 ans, femelle 2 ans)	1 naissance en en forêt, en bonne santé (mâle 1,5 ans)	0

Comportement

- budgets temps similaires à ceux observés chez les populations sauvages,
- Construction de nids
- Groupes sociaux
- Chimpanzés en mode fusion fission, les femelles se mêlent à des groupes de sauvages
- Les gorilles restent dans leur groupe de départ jusqu'à l'adolescence ou les mâles deviennent solitaires
- Autonomie alimentaire
- (138 espèces consommées pour les chimpanzés, 60 pour les gorilles)

Utilisation du territoire

- Exploitation du milieu
- Gorilles 4 km2 en saison des pluies et 1,34 km2 en saison sèche
- Chimpanzés: plus de 30 km2
- Les gorilles mâles adolescents parcourent de très grandes distances; un second site de relacher a été choisi au PPG Congo, et les groupes déplacés: Réserve de la Léfini: 47 km2 de forêt galeries et 53 km2 de forêts associées

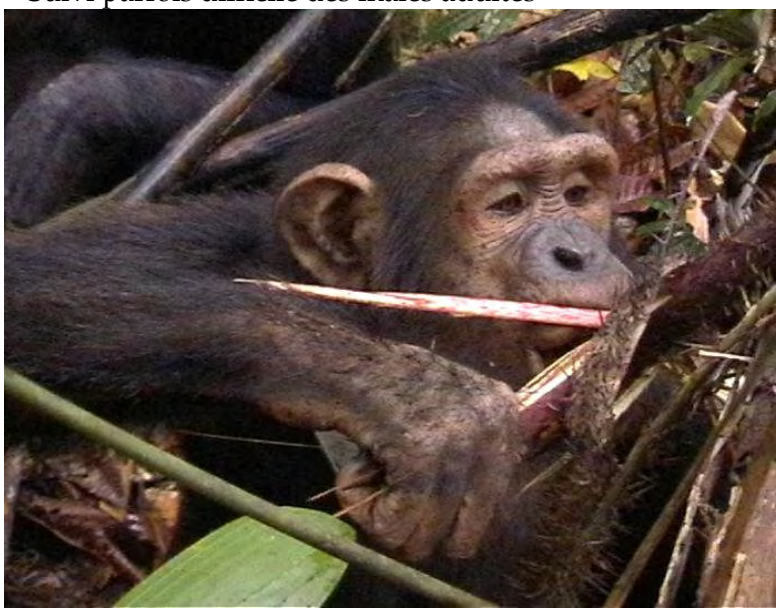
Protection du milieu

- Participation effective à la lutte anti-braconnage (et observation de l'augmentation des indices de présence de la faune sauvage)
- HELP: Aide au développement de ressources de substitution (plantation 2500 arbres)
- Actions de sensibilisations par le biais de films, expositions, animations dans les écoles

Les difficultés rencontrées

- Gorilles
- Très sensible au stress
- Grande mortalité au départ
- Détachement de l'homme difficile
- Tolèrent mal les inconnus: contraintes pour le personnel
- Les mâles solitaires étendent leur territoire plus que prévu: risque d'atteindre des habitations
- Difficile de suivre les groupes à partir de l'adolescence

- Chimpanzés
- Beaucoup d'attaques par les chimpanzés sauvages (surtout mâles et bébés). Le nombre de morts serait supérieur sans une présence vétérinaire permanente
- Fuite des individus lors du relâcher
- Suivi parfois difficile des mâles adultes



Les facteurs de réussite

- Site de relâcher approprié
- Constitution de groupes d'affinité avant le relâcher
- Choix des individus
- Âge: plus difficile pour les adultes
- Sexe: plus difficile pour les mâles
- Plus difficiles pour les individus « imprégnés »

- Un personnel qualifié et nombreux, surtout les 2 premières années

Discussion

- A l'heure actuelle, les populations sont viables et autonomes
- Les zones de relâcher sont effectivement protégées
- Quel nombre d'individus faut-il réintroduire pour avoir une population viable sur de nombreuses générations ?

5.4. AN OVERVIEW OF RESPIRATORY INFECTION OUTBREAKS IN CHIMPANZEES AT THE LIMBE WILDLIFE CENTRE

J Kiyang, (LWC)

SIGNALEMENT

- Out break in 2006 and 2007
- Out break in a population of 40 chimpanzees.
- Chimps of all ages, sex and subspecies involved
- Outbreaks occurred in the wet months of the year (May to August).
- Other primates did not show same susceptibility
- Some cases mild and resolved without chemotherapy
- Other cases moderate and required tx
- Some cases potentially fatal, required aggressive tx
- Fatality rate low with aggressive & timely tx

CLINICAL PICTURE

Figure 1. Many cases showed severe catarrhal buildup

TEMPERATURE

- Range from 38°C to 40°C
- Decreased to 34.60C, shocked.

RESPIRATORY TRACT

- Range from serous to mucoid, thick catarrhal nasal discharge
- Catarrh caking at nares (Figure 1)
- Congestion of the bronchus/bronchi and nares
- Dyspnea
- Tachypnea
- Ausculatation - fluidy to sticky sounds

SEVERE CASES

- Dry unproductive initially, then wet productive in prolonged cases
- Progresses to suppressed cough with signs of pain

- Depressed and lethargic
- Weak and recumbent
- Anorexia
- Hydration: moderate to severed dehydration
- Blood Picture
    - mucus membranes: pale pink
    - PCV: < 30%
    - CRT: 2 secs to 3 secs.
    - Total WBC: > 20 x109/L
    - Left shift neutophilia

THERAPY

Figure 2. General anaesthesia was required to administer administration of medications in severe cases

- Uncomplicated cases – no treatment
- Moderate cases – antibiotic treatment (TMS or clav-amox)
- Severe cases – General anaesthesia (then maintained on Diazepam) to enable IV treatment (Figure 2)
    - **IV then IM Ceftriaxone or IM Baytril**
    - **Anti-inflammatory – Metacam useful for pain relieve**
    - **Expectorants – Carbocisteine helpful in all, especially babies**
    - **Topical and nebulisation of Vicks VapoRub® where convenient to use**
    - **Ranferon (blood building tonic) used as supportive treatment**
    - **Antifungal (Nystatin) to prevent fungal overgrowth**
    - **PHARYNGEAL SWABS IN RNA LATER TAKEN FROM ALL SEVERE CASES**

Year	symptomatic	Moderate	Severe	Treated	Recovered	Died
2006	36	30	6	36	36	0
2007	23	18	5	22	22	1

Case Study: GABON (Figure 3 and 4)

Figure 3 and 4 – Gabon, a young chimp wioth severe respiratory infection.

Laryngopharynx swab results 2006 outbreak

Name	Sample	HMPV	S.pneumonia
Jack	Swab	Neg	Pos
Jack	Swab	Pos	Pos
Bankim	Swab	Pos	Pos
Mesang	Swab	Pos	Pos
Taweh	Swab	Pos	Pos

Laryngopharyngeal swab results for 2007 outbreak

Name	Sample	RSV	S.pneumonia
Bergkamp	Swab	Pos	Pos
Gabon	Swab	Pos	Pos

DISCUSSION

HRSV and HMPV:

- Paromyxovirus, worldwide distribution, humans are definitive host.
- Self limiting upper respiratory tract infection in adult primates
- Fatal cases known in human infant and great ape
- In combination with S pneumonia and/or P multocida led to fatal infection *(Sophie kontgen et al, Current Biology, Feb 2008)*

Source of infection:

- Humans – staff. visitors

Preventative strategies:

- Keepers use masks
- Stay away when sick
- Visitors no contact
- No offering of food to animals by visitors
- Good distance between visitors and apes, etc

SIGNIFICANT RISK TO WILD APE POPULATIONS IN CONTACT WITH HUMANS?

- HABITUATED APES eg. Mountain gorillas
- RESEARCH GROUPS eg Tai Forest chimps
- HABITAT ENCROACHMENT eg. Cross River gorillas

CONCLUSIONS

- LWC EXPERIENCE HAS SHOWN THERE IS A SPECTRUM OF SEVERITY
- NOT ALL CASES NEED TX
- MOST NEED AB TX
- NEED TO MONITOR ALL CASES TO IDENTIFY THOSE ANIMALS THAT ARE DEVELOPING SEVERE PNEUMONIA. THESE CASES REQUIRE AGRRESSIVE INTERVENTIONS DESCRIBED (GA, IVF TX, IV ANTIBIOTICS ETC).
- STRICT PREVENTATIVE MEASURES NEED TO BE ENFORCED TO REDUCE DISEASE TRANSMISSION BETWEEN HUMANS AND APES IN BOTH CAPTIVE AND WILD ENVIRONMENT

5.5. L'INFECTION DU SAC LARYNGE CHEZ LES BONOBOS DU SANCTUAIRE LOLA YA BONOBO (Kinshasa, DRC)

C Mahamba, (Lola Ya Bonobo)

CHRONOGRAMME

- Genèse d'un cas clinique: MAYA;
- Extention de l'infection à d'autres bonobos;
- Deux formes d'après l'évolution;
- Recherche de solutions: 4 modes d'intervention;
- Pyocultures et antibiogrammes;
- Discussions
- Conclusions
- Remerciements

POSITION DU SAC LARYNGE

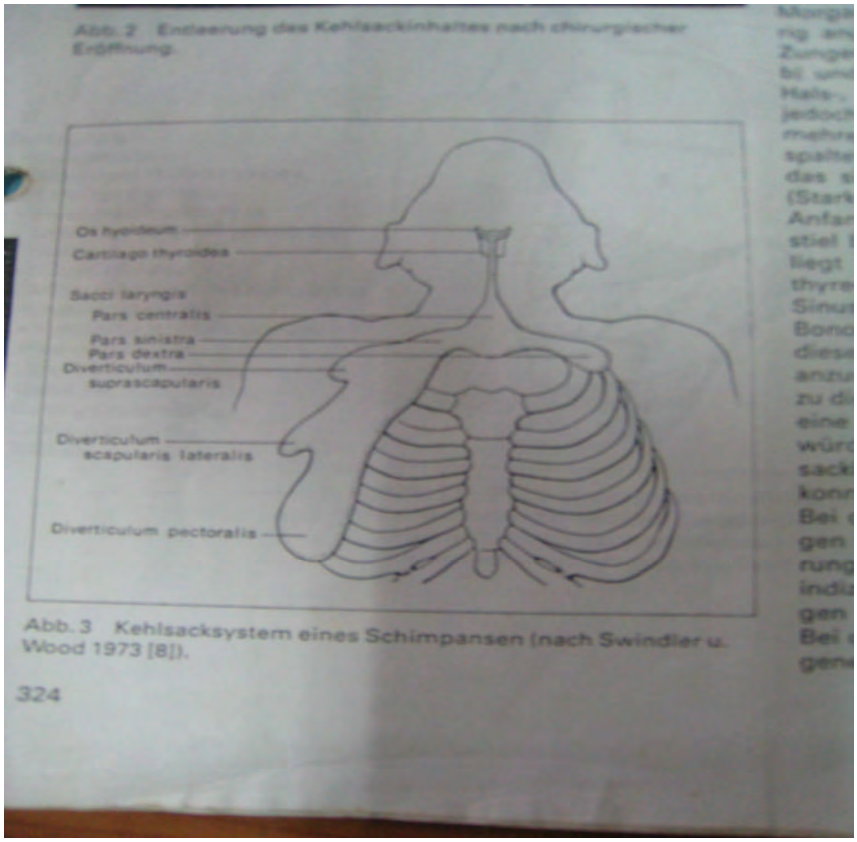


Abb. 3 Kehlsacksystem eines Schimpansen (nach Swindler u. Wood 1973 [8]).

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DIVERSES FONCTIONS

- Hyperventilation
- Vocalisations
- Call rate
- Call duration
- Resting breathing rate

DISTRIBUTION

- Espèces classées selon la présence ou l'absence du sac laryngé:

- Strepsirhinae: 2 espèces
- Colobinae: 11 espèces
- Hominoidea: 4 espèces
- Cebidae,Callitricidae : 10 espèces
- Cercopithecinae : 29 espèces

CAS CLINIQUE

- MAYA
- Femelle de 7ans pésant 19kgs
- Arrivée au sanctuaire en 1995
- **Août 1999**: gonflement du sac laryngé rempli d'air;
- **Decembre 1999**:
  - contenu liquide
  - l'état général n'est pas affecté

JANVIER 2000:
Début d'infection(fièvres (38-38,8°c),douleur et rougeur): contenu purulent; - Extraction du pus par seringue;
Pyoculture: Streptocoques hemolytiques B
Antibiogramme: sensibles (ampicilline, erythromycine,céfalexine et chloramphénicole);
Traitement: Erythromycine250mg/5ml : 500mg/3fx/j 7jrs;
Résultat: Accalmie de courte durée (4,5 mois).

MAI 2000:
Réinfection(contenu purulent, fièvres(38,4-39°c),mauvaise haleine et anorexie);
- Intervention chirurgicale par Dr Nick de Souza (WSPA);
Soins post-opératoire: Céfalexine 250mg/5ml: 500mg/3fx/j 10jrs;
Résultat: Accalmie plus ou moins durable (10 mois).
Mode opératoire
1. Animal en position couchée,tête mi-levée;
2. Rasage et désinfection;
3. Incision longitudinale du sac laryngé (2,5 cm) et collecte du contenu purulent (96ml);
4. Nettoyage avec 1g de Rocéphine + 1/2 litre de NaCl 0,9%;
5. Suture avec du fil résorbable(vicryl 0-2) et Rocéphine 1g en IM + du Métacam (analgésique)
6. Poursuite de soins avec du céfalexine oral 250mg/2fx/j pendant 7jrs.

MARS 2001:
Début du gonflement du sac laryngé(contenu liquide);- Infection respiratoire (bronchite: toux muqueuses, fièvres(38-39°c);
Traitement: Chloramphénicole 250mg/5ml: 500mg/3fx/j 10jours;
Résultat: Accalmie plus ou moins durable (11mois)

FEVRIER 2002:
Réinfection du sac laryngien(contenu purulent volumineux (poing de main); - Infection respiratoire(broncho-pneumonie: douleurs, haleine putride, fièvres(38,5- 39,6°c) et cyanose);
Traitement: Céfalexine 250mg/5ml: 500mg/3fx/j 10jrs;

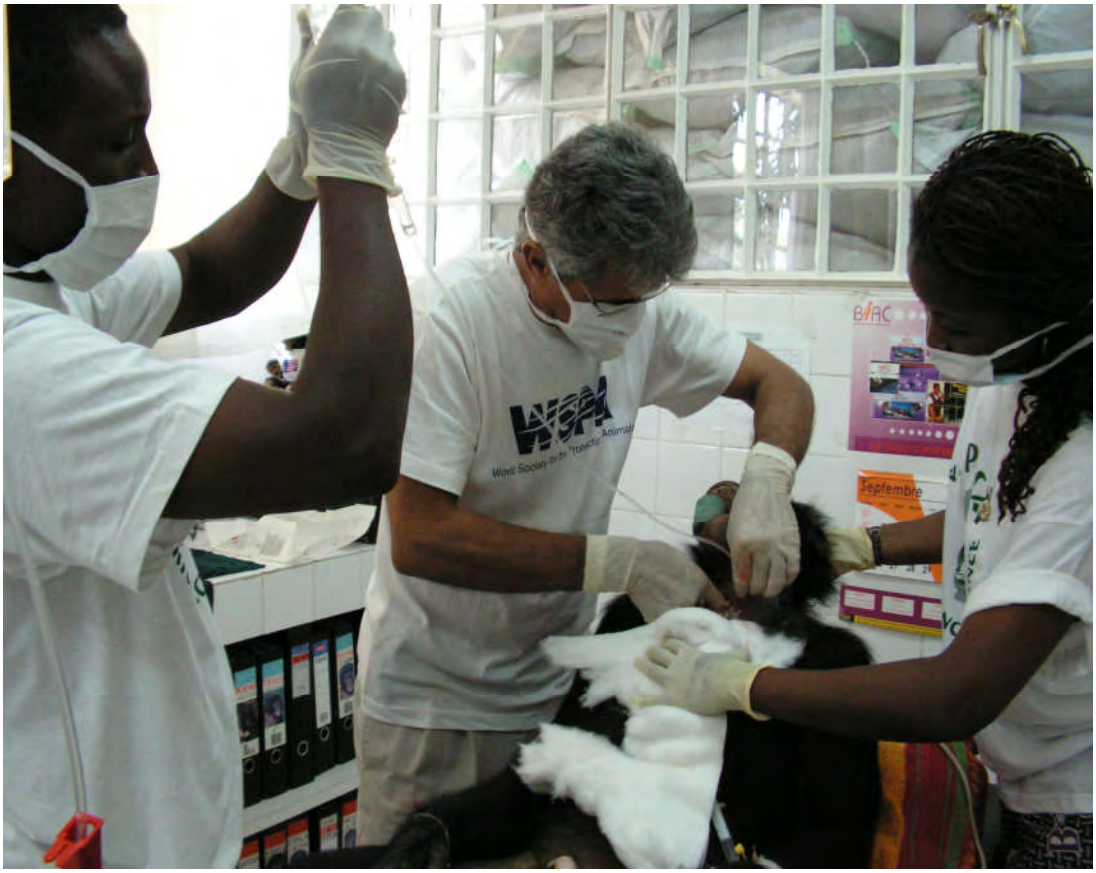
Résultat: bon et durable(broncho-pneumonie), bon et court pour l'infection du sac laryngé (4mois).

JIUN 2002 :
Retour de l'infection : contenu purulent et fièvres(38-39,2°c); - Deuxième intervention chirurgicale par Dr Nick de souza (WSPA)
Pyoculture: Stréptocoques hémolytique B;
Traitement: Idem première intervention;
Résultat: accalmie plus ou moins durable (11mois)

MAI 2003 :
Retour de l'infection(contenu purulent moins volumineux) accompagnant une grippe(rhume, toux et fièvres de 38-39°c);
Traitement: céfalexine : 250mg/5ml : 500mg/3fx/j 7jours;
Résultat: réponse de courte durée (4mois).

OCTOBRE 2003 :
Réinfection : contenu purulent, fièvres(37,9- 39°c) et mauvaise haleine); - Troisième intervention chirurgicale par Dr Nick: ablation du sac laryngien. Mode opératoire çi-dessous.

MODE OPERATOIRE:
1. Préparation pré-opératoire (même procédure);
2. Incision longitudinale (6cm) et collecte du contenu purulent (126ml)
3. Ablation du sac laryngé: incision dans sa partie frontale et suture du moignon(vicryl 1-0 résorbable)
4. Suture de la plaie opératoire avec du fil non résorbable(ETHICON 1-0);
5.Traitement: 1g de rocéphine IM completé par du Céfalexine oral 250mg/2fx/j pendant 7jrs;
6. Les files de sutures sont enlevés 7jours après.
1ère Intervention chirurgicale de Maya par Dr Nick de Souza (WSPA).



INQUIETUDES

1. Depuis lors, plusieurs bonobos du sanctuaire ont été atteint par l'infection:
   Tatango (mâle de 15 ans),
   Opala (femelle de 14 ans),
   Matadi (mâle de 8 ans),
   Kalina (femelle de 12 ans),
   Mixa (mâle de 8 ans),
   Mbandaka (mâle de 7 ans)
   Tembo (mâle de 12 ans).

2. Des nombreux cas de récidives après les interventions chirurgicales identiques aux deux premières de Maya ont été observés dans une période allant de 2 à 10 mois.

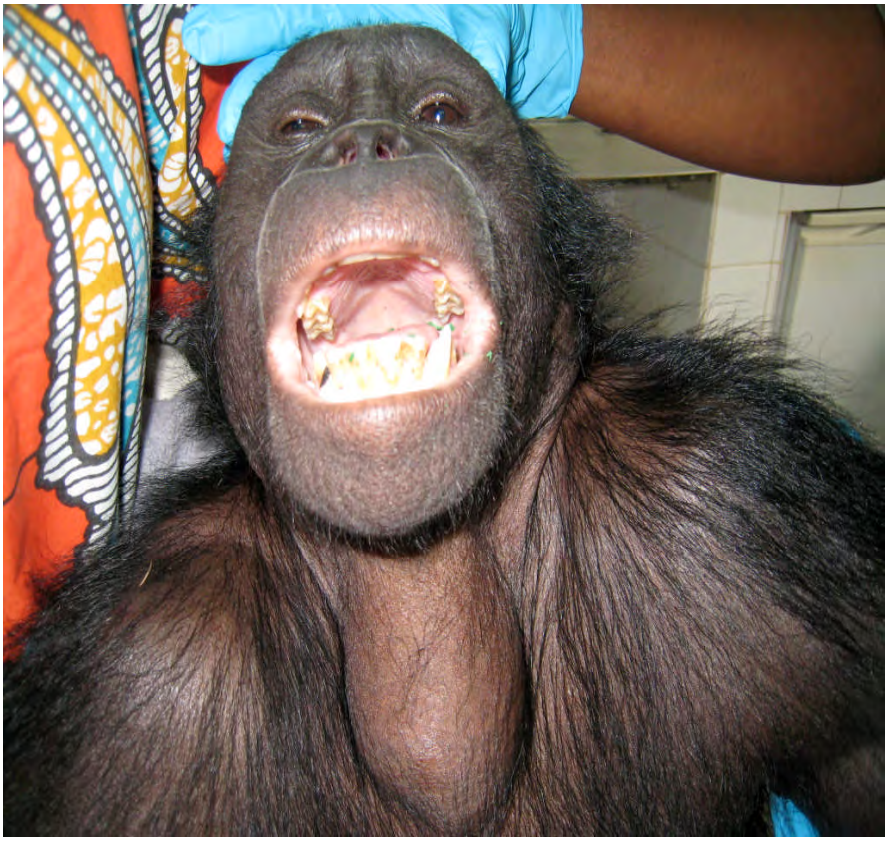
L'EVOLUTION DE L'INFECTION

Distinction de **deux formes** :

1. Forme en tube

Ici on observe une inflammation et stockage de pus tout au long du larynx.

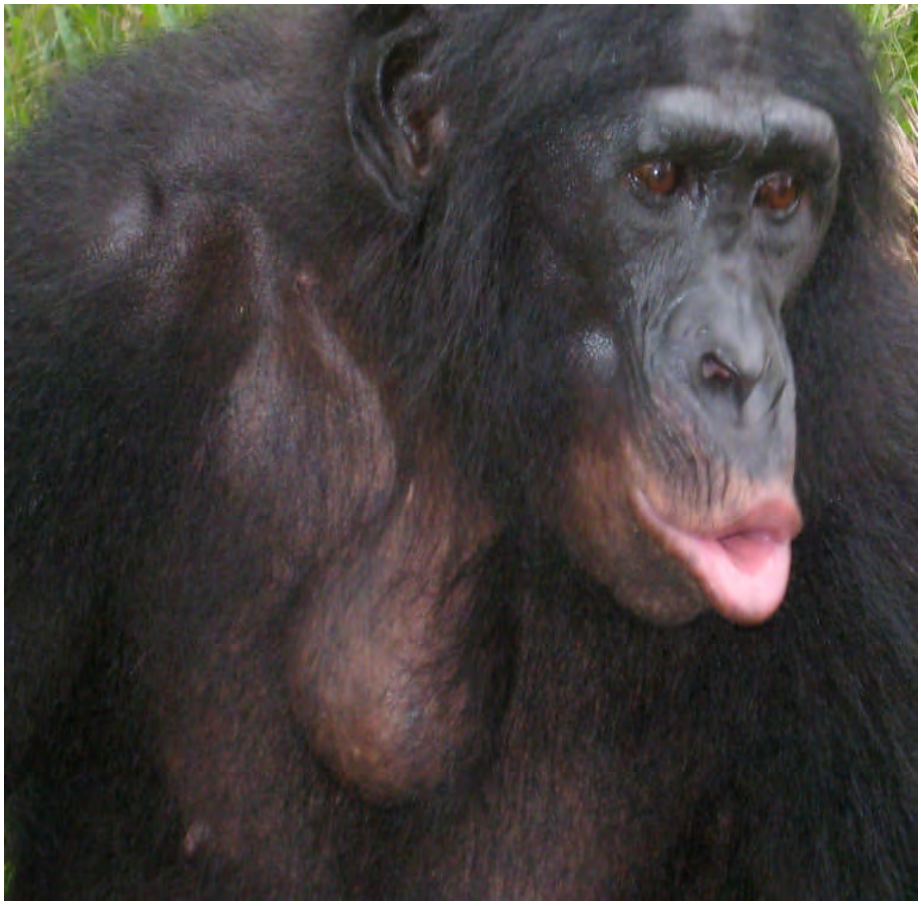
L'évolution de cette forme est très rapide et très douloureuse exigeant une intervention rapide en quelques jours (moins de 7jours)



2. Forme en Calebasse:

Ici on observe une accumulation d'exsudat purulent en position declive dans la partie ventrale de la poche.

L'évolution est lente et moins (ou pas) douloureuse. L'animal peut vivre normalement plusieurs mois sans se plaindre.



RECHERCHE DE SOLUTIONS

1. Aspiration de l'exsudat purulent par une seringue (60ml) et une aiguille (18G 11/2).

Résultat:

Réformation du pus dans la poche dans un délai court compris entre 2 et 6 mois.

2. Ouverture du sac laryngien, nettoyage et suture complète (fermeture totale du sac)

Résultat:

Réinfection dans un délai compris entre 4 et 10 mois de l'opération.

3. Ablation du sac laryngien infecté et ligature du moignon(fil résorbable 1-0 vicryl)

Résultat:

Solution durable, mais on observe une faible réduction de l'amplitude du son:

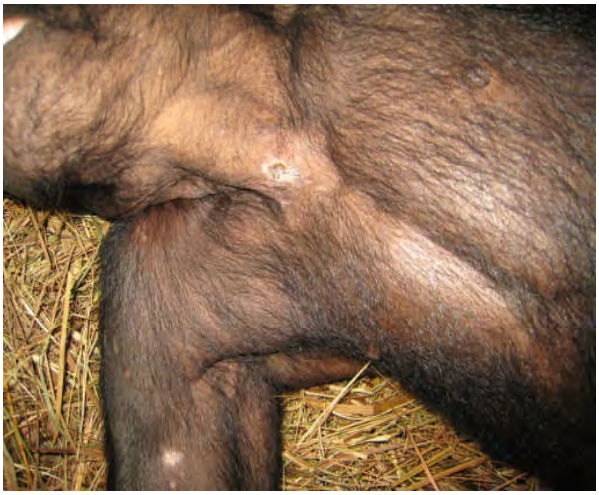
L'animal vocalise plus bas que d'habitude (expérience personnelle vécue au sanctuaire de Kinshasa).

Ouverture du sac, nettoyage et suture incomplète: Un petit orifice de drainage de pus est laissé en place.

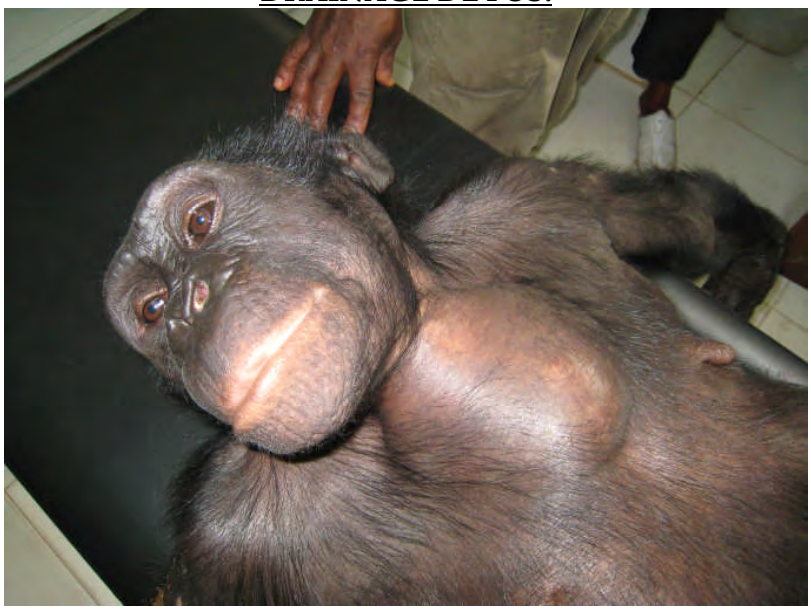
Résultat:

Satisfaisant pour les périodes d'observations suivantes:

1. Tembo: 7 mois
2. Matadi: 5 mois
3. Opala: 3 mois.

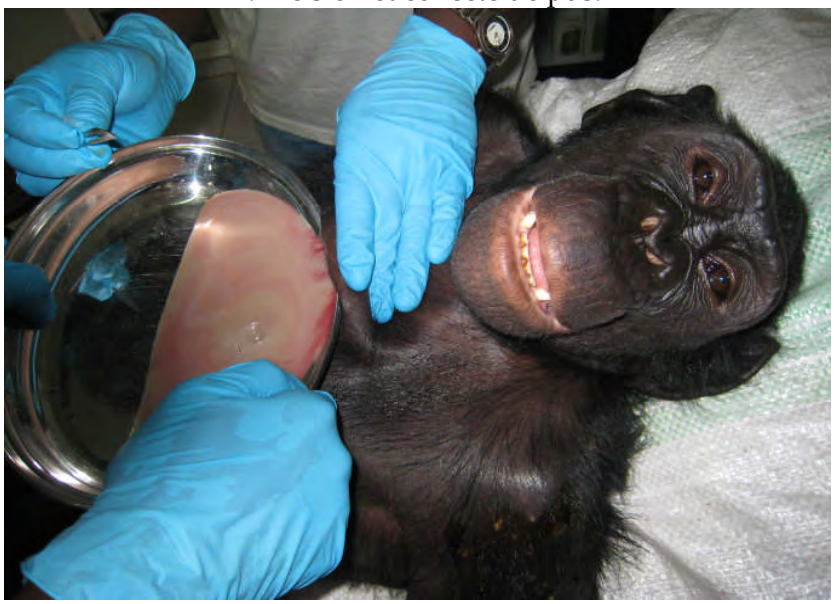


PROCEDURE DE L'OPERATION LAISSANT EN PLACE UN ORIFICE DE DRAINAGE DE PUS.

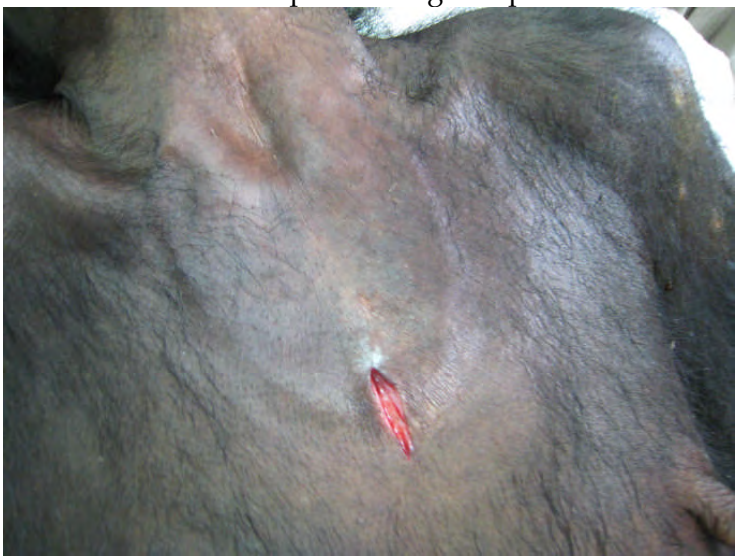


1. Rasage et désinfection.

2. Incision et collecte de pus.

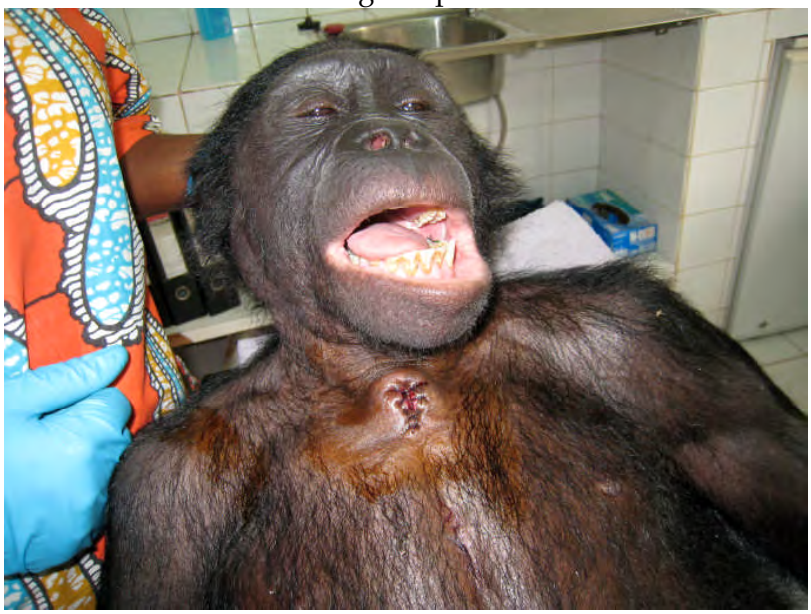


3. Plaie après vidange du pus.

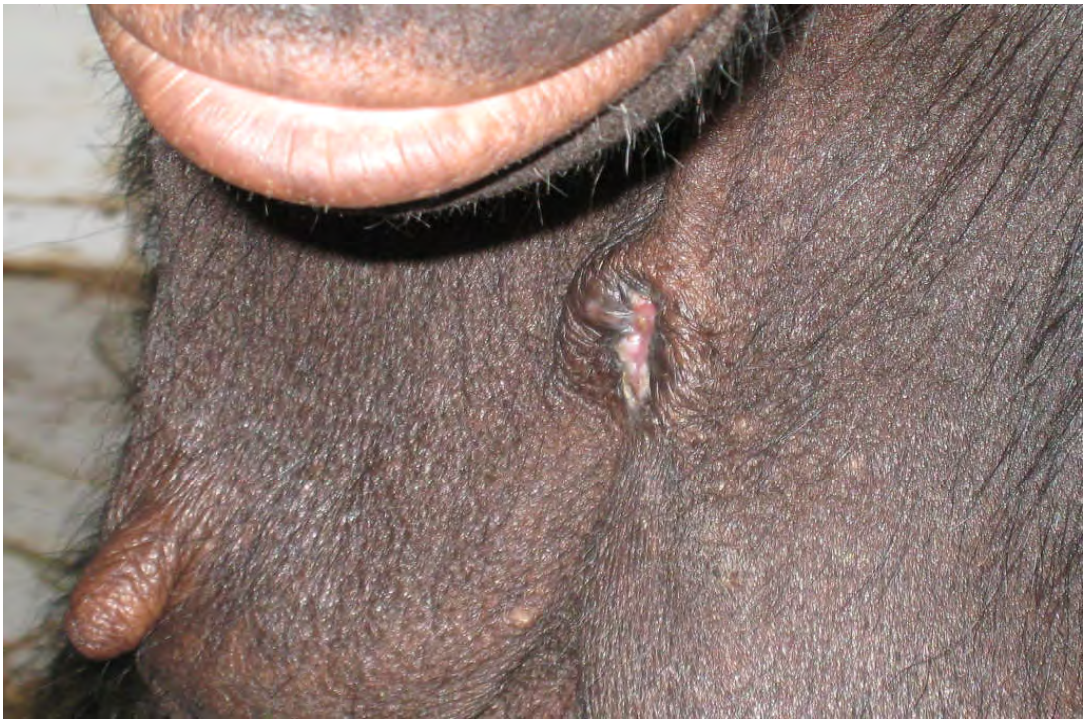


4. Nettoyage et suture incomplète.

5. Un orifice de drainage de pus suturé tout au tour.



6. L'orifice de drainage 3 mois après l'opération.



RESULTATS D'ANALYSES DU PUS AU LABORATOIRE

- **Maya**: *Stréptocoque hemolytique B*
- **Mbandaka**:*Staphylocoque aureus*
- **Matadi**: *Escherichia coli*
- **Tembo:** *Escherichia coli*
- **Tatango**: *Escherichia* coli, *Klebsiella sp.*
- **Opala:** *Staphylocoque aureus, Escherichia* coli

RESULTATS ANTIBIOGRAMME

Degré de sensibilités aux produits:

1. *Streptocoque hemolytique B*

Sensible	Résistant
Ampicilline	Penicilline
Céfalexine	Oxacycline
Tétracycline	Kanamycine
Erythromycine	Polamine
Chloramphenicol	**10.1**

2. *Escherichia coli*

Sensible	**Résistant**
Aminosides	Phenlolilines (penicilline)
Chloramphenicol	Tetracycline
Quinolones (Ciprofloxacine, Négram)	Doxycycline
Céphalosporines	**0.2**

3. *Klebsiella spp.*

Sensible	**Résistant**
Aminosides	Phenlolilines (penicilline)
Chloramphénicol	Céphalosporines
Norfloxacine	Tétracycline
Ciprofloxacine	Négram

DISCUSSIONS

L'infection du sac laryngien a dans la plupart des cas, accompagné un problème respiratoire (rhumes, bronchite ou broncho-pneumonie) comme cela a été reporté ailleurs (Gorille, Orangutan et Bonobo).

Nous ne savons pas ce que pourraient être les conséquences à long terme de l'ablation du sac laryngien; Bien que le bonobo opéré au sanctuaire de Kinshasa se porte très bien depuis

Mêmes résultats dans d'autres centres:

- Zoo de Wuppertal (Stuttgart, Allemagne):
- Le germe isolé est ***Streptocoque hémolytique b*** sur un bonobo de 7 ans.
- Zoo de Philadelphie (USA):
- Le germe isolé est ***Eschérichia coli*** sur un Orangutan de 14 ans.
- Centre de recherche Karisoke (Rwanda):
- Le germe isolé est ***Staphylococcus aureus*** sur un gorille de montagne de 31 ans.

Quant à MAYA qui a perdu totalement son sac laryngien, nous avons noté tout simplement une faible diminution de l'amplitude lorsqu'elle émet un son de haute fréquence.

Pour le reste, elle se porte très bien et n'a plus connu une infection de ce genre depuis 2003.

Les trois bonobos (Tembo, Matadi et Opala) ont connu des cas de récidives. OPALA a en plus connu une réinfection après avoir arraché les files de suture après son opération de mai 2008. Ne les ayant plus enlevés après l'intervention de septembre 2008, elle évolue très bien aux côtés des deux autres.

L'évolution de la guérison est apprécié par palpation tout les deux mois partant de la date d'intervention chirurgicale.

QUESTIONS

- L'intervention chirurgicale laissant en place un orifice de drainage de pus, ne peut-elle pas favoriser l'entrée et le développement d'autres microbes?

- Comment prévenir l'infection du sac laryngien dans le contexte des sanctuaires?

- Existent-ils des facteurs favorisant l'infection? Car sur un cohorte de 62 bonobos, seuls 8 l'ont eu.

CONCLUSIONS

1. L'infection de sac laryngien ne révèle pas un caractère contagieux au sanctuaire de kinshasa.
2. La solution ultime est l'intervention chirurgicale évitant l'accumulation ou passage du pus dans le champ pulmonaire.
3. Les résultats sur la technique de drainage post-opératoire sont préliminaires au sanctuaire « Lola ya Bonobo » et seront appréciés dans un an.

5.6 CREATING A FIELD BACTERIOLOGY LAB

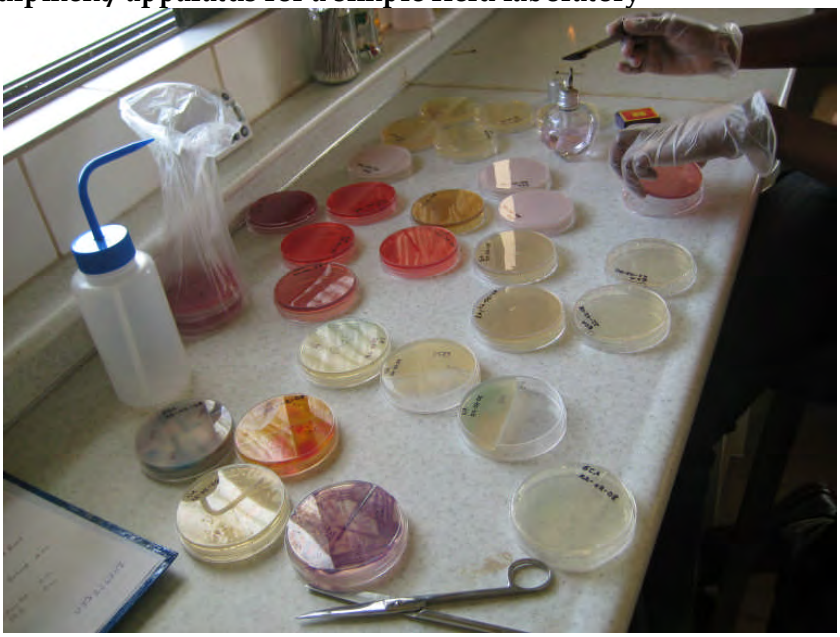
F. Nizeyimana, Ngamba Island Chimpanzee Sanctuary and Wildlife Conservations Trust (CSWCT)

PRACTICAL MICROBIOLOGY

Microbiology aspects under consideration (Ngamba Island Chimpanzee sanctuary/ Field interventions)
Field intervention laboratory procedure

- Sample collection
- Sample preservation and transport
- Culturing of bacteria in the collected samples
- Picking of the colonies
- Preservation of colonies/ stabbing
- Packing/Shipping of the stabs

The equipment/ apparatus for a simple field laboratory



Microbiological plates with agar;Shigella-Salmonella agar,McConkey agar ,Muller Hinton agar,XLD,TSI/Slants needed for use

Universal tubes for samples

Tooth picks (Gamma irradiated)
Cryotubes or vials for stabs (with transport media e.g. PEG)

Media for preservation

- Sorbitol
- Glycerol
- Peptone water agar

Incubator(portable) and thermometer

Disinfecting equipment

- Paper towels
- Hand gel/99.9% germicide disinfectant
- Ethanol (99.9% and 70%)
- RNA later (for tissue samples)
- Applicator sticks, Tongue depressants
- Gloves (disposables)
- Bins/disposal bags for biohazard materials
- Ziploc bags

Storage, preservation, transport equipment

- Markers(fine or extra fine point)
- Boxes and packing bags(transport)
- Refrigeration for Media(cool boxes for field or gas fridge for island)
- Sample tubes(universal tubes of 20ml, 10ml, 5ml, 1ml, large, small, medium)
- Cryotubes(0.5ml, 1ml)
- Recording paper
- GPS
- Stop watch/wrist watch
- Field kit/ box

Samples of interest

Should be freshly collected

- Dung/ faeces
- Urine
- swabs(anal)
- Water samples

Method of collecting samples

- Freshly collected as and when being passed out (faeces and urine) for the non-human primates
- per rectum(faeces) for anaesthetised primates and domestic animals
- manipulating of abdomen (urine) in anaesthetised primates and domestic animals

Culturing of bacteria in the samples after collection
- Streaking using the tooth picks
- Labelling using fine point markers
- Incubation between 18-24 hrs(Using portable incubator)
- Incubation at 37˚Celsius**,** the incubator should be moistened

Preservation of bacterial colonies grown
- Preservation by stabbing onto media in 0.5ml Cryotubes
- Labelling of the stabs
- Packing of stabs in 0.5 ml Cryotubes

N.B: Over grown colonies should not be picked, stabs should be from clearly defined colonies.

Disposal of lab waste and used plates
- Pit latrine is helpful for the field but the plates should be autoclaved first.
- Disposal should be done in labelled biohazard bags/ containers on site and these taken to the mainland (hospital or UWEC). Ensure that these are properly disposed of.

No Bunsen burner; due to this the following are required:
- Disposable gloves, sticks, paper tissue and towels
- Already prepared media

Bacterial colonies growing on the media observed the following day
- For example *E.coli*; the colony characteristics include:
    - Round and smooth
    - Small-medium size
    - Reddish-Pinkish

Pick clearly defined colonies

Re culturing to get isolates for preservation; Should be done to get clearly defined colonies using the picks then stabbed onto media for preservation.

5.7 OCULAR AND SURGICAL CASES FROM OL PEJETA CONSERVANCY

A Magre, MRCVS, CertVOpthal. Vet Advisor to Ol Pejeta

EYE PROBLEMS

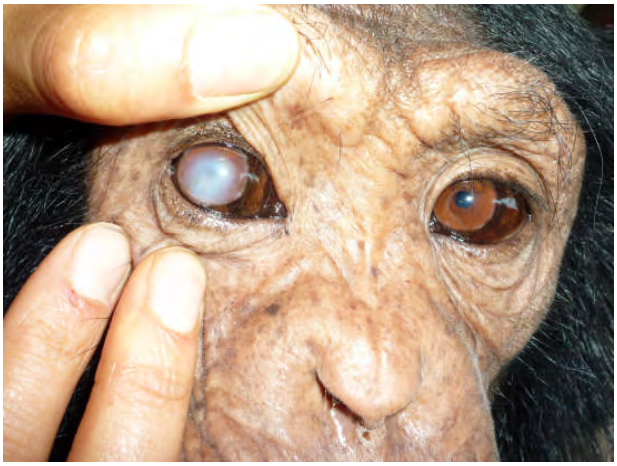
- 5 males with one or both eyes affected
- Aged from 3 to 27 years
- Complete ophthalmological examination starting 2007
  - Schirmer Tear Tests
  - Pentorch
  - Fluorescein staining
  - Slit lamp biomicroscopy
  - Tonopen
  - Complete Blood Analysis
  - Corneal and Conjuctival Swabs

Findings

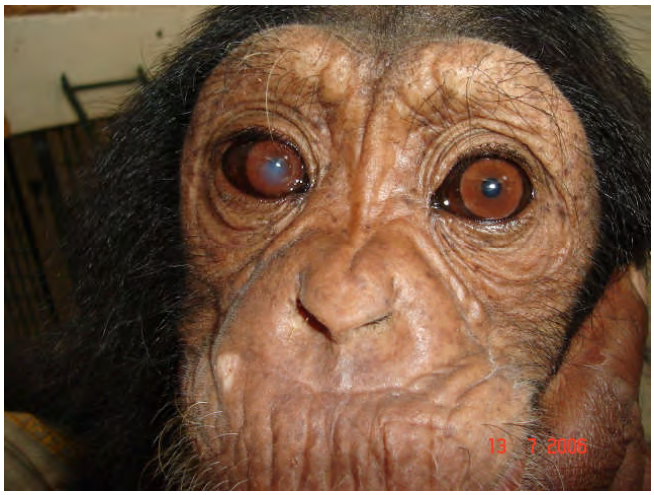
- STT normal
- IOP normal
- Fl –ive (except Romeo and Uruhara)
- Corneal lesions – some lipidosis and corneal scarring (except Romeo and Uruhara)
- Blood – All normal
- Conjuctival and corneal smears consistent with squamous metaplasia of the conjuctiva

Romeo

- Corneal ulcer shallow Fl+ive
- Corneal oedema surrounding
- Some superficial corneal blood vessels to ulcer edge
- Superficial grid keratectomy
- Fucithalmic eye drops bid
- Deep corneal scraping taken and fungal hyphae identified
- Miconazole eye drops 6 times a day + Fucithalmic bid for 8-12 weeks

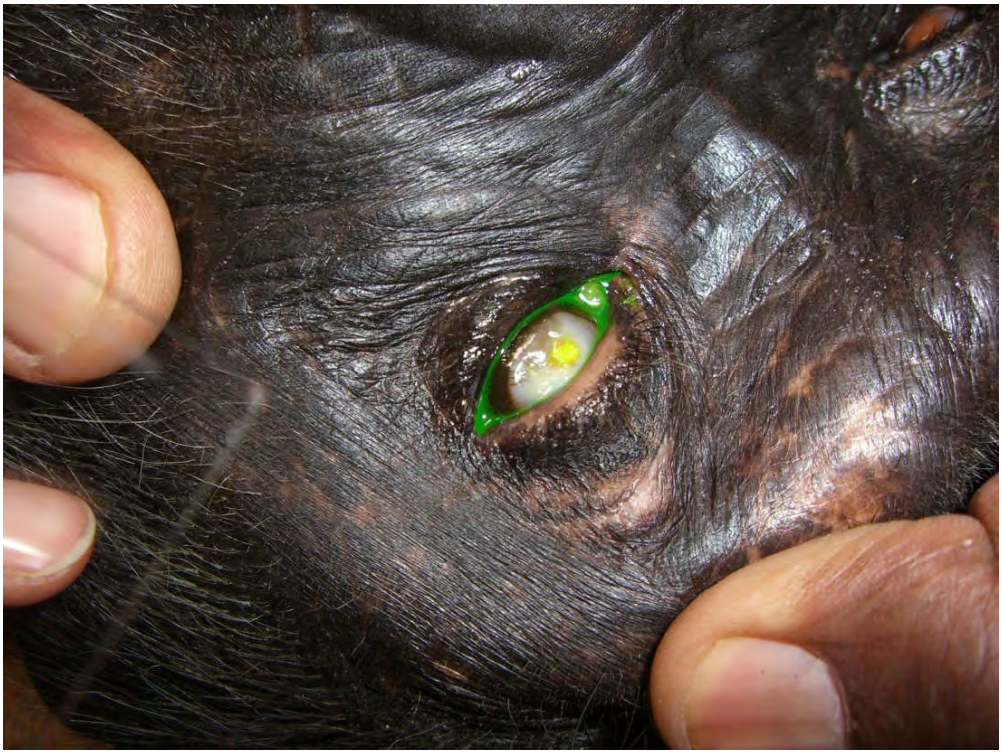


Ulcer healed totally with some residual corneal scarring which should regress with time.



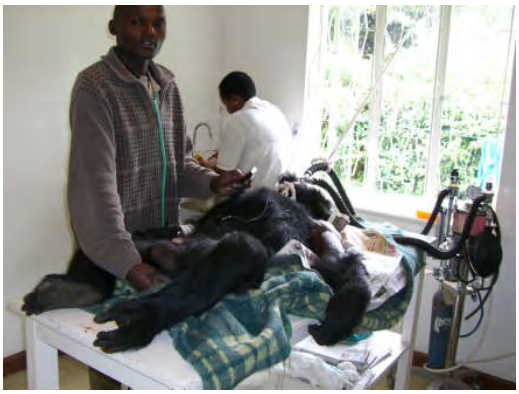
Uruhara

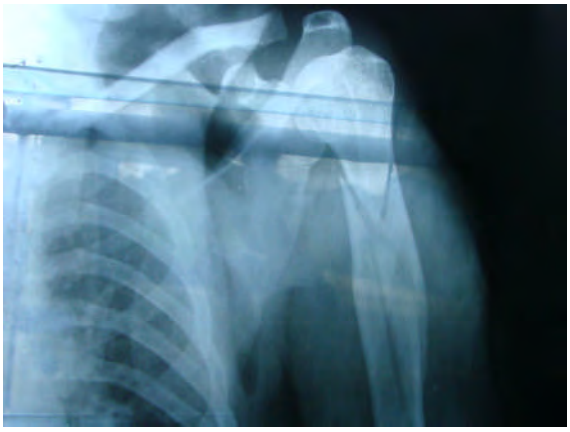
- Bilateral eye problem
- Right eye – some corneal scarring – non progressive
- Left eye – corneal ulcer with calcium deposits
- Photophobia
- Left eye worsening
- Not much response to fucithalmic

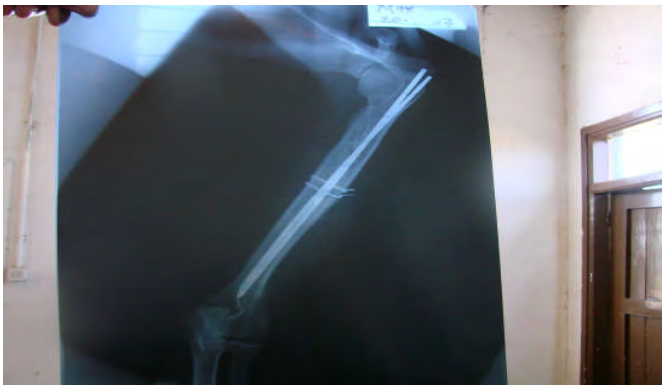


FRACTURE – LEFT HUMERUS - MAX

- Fracture of the left humerus – Long transverse
- Proximal 1/3
- Required intramedullary pins and cerclage wire
- Done under general anaesthetic
- Recovery uneventful – restricted exercise in a cage for 10 weeks then in a room for 4 weeks, then allowed out for short periods of time and gradually increasing
- No problem with use of the arm – pins have not protruded, so are still in the bone

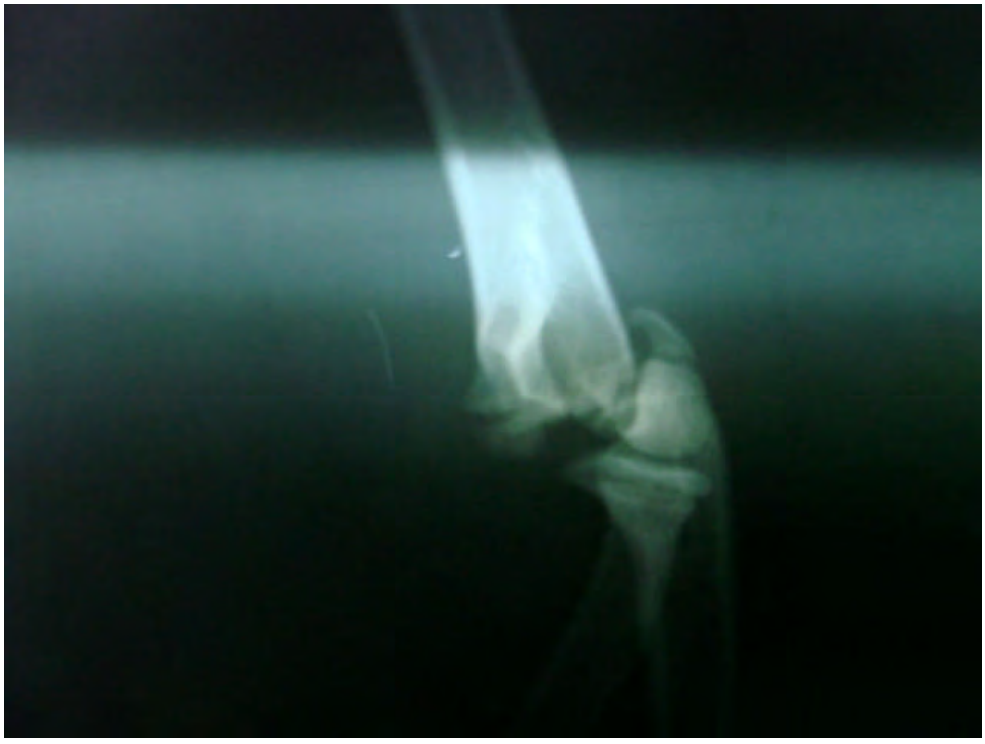


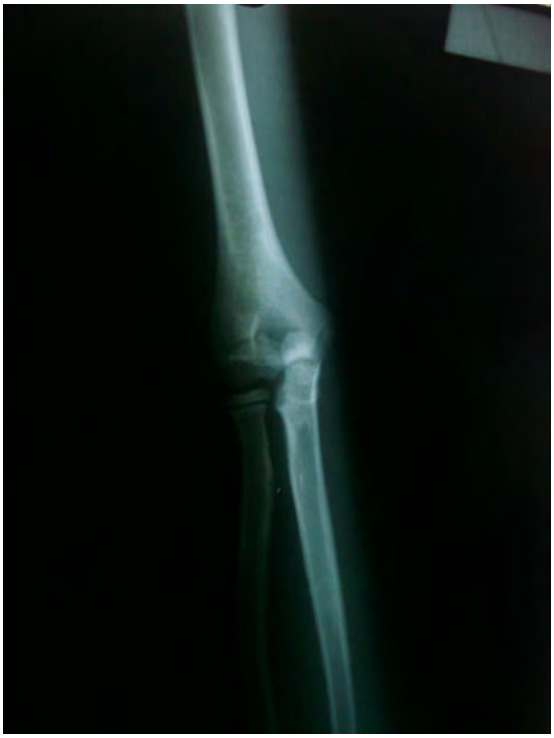




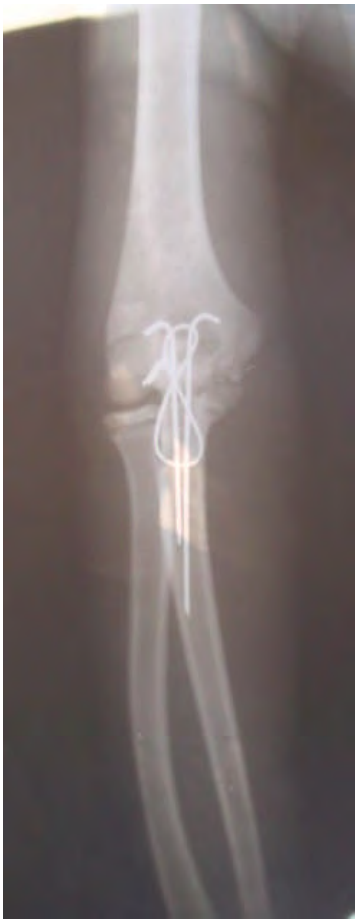
FRACTURE – LATERAL CONDYLE AND DISLOCATION OF THE ELBOW - GEORGE

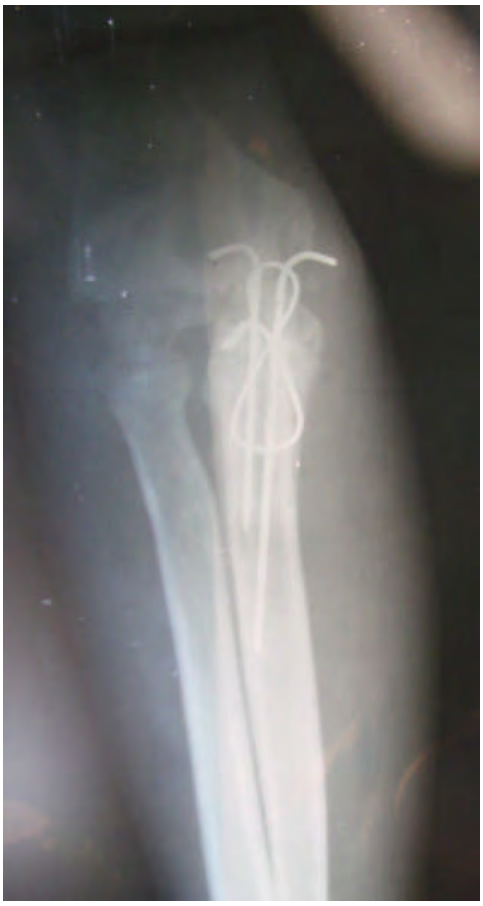
- Dent fracture of medial epicondyle
- 30% - 40% of articular surface cartilage of distal humerus crushed
- No significant piece of bone found
- Olecranon displaced from olecranon fossa
- Operation done under GA





- Joint reduced with good range of motion
- Olecranon fixed using tension band wiring method
- Joint checked and found to be stable and in place
-





5.8 VETERINARY PROTOCOLS AND PROBLEMS IN PREPARATION FOR THE RELEASE OF 12 CHIMPANZEES

C Colin, Centre for Chimpanzee Conservation, Guinea

- Release planed for June 2007
- Veterinary preparation in Oct/Nov 2006
- Political troubles in Guinea Jan/Feb 2007 postponed the release until 2008. Release occurred 27th June 2008.

12 chimps:

6 males and 6 females (2 captive born, with their mum); 8 years old to 20 years old

- 4 males moved in March to the released site (=real quarantine for them)
- 1 male moved 3 weeks before release
- 7 chimps moved the day of the release

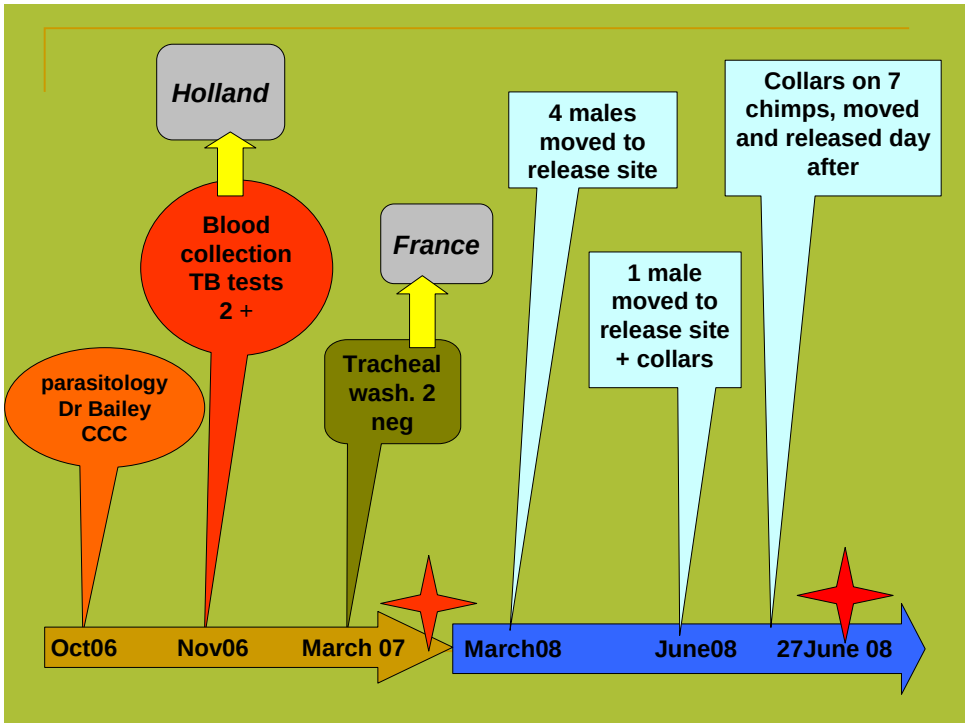


Figure 1. Overview of the Testing Regime

Parasitology

- All chimps tested in October 2006 (Dr W. Bailey, Senior Parasitologist, Liverpool School of tropical Medicine), as well as CCC staff (Figure 2)
- Results negative for pathogenic parasites
- No more done in 2008, but SHOULD HAVE BEEN DONE (lack of time, organization on site: vets occupied with non-vet problems)
- Chimps received ivermectin before release

Figure 2. Dr Bailey with CCC staff conducting parasitological tests on the release group.

Blood tests

- Blood collections done in November 2006 (1 week)
- Blood sent in Europe in December for analysis (Holland)
- Problems with transport (time needed) - conservation prior and during transport
- No more done after (logistics, opportunities, etc.)
- No significant findings (Testing followed Disease of concern protocols)

TB tests

- Done in November 2006, MOT
- ***2 chimps positive (both eyes swelling)***
- *Tracheal wash* for these 2 chimps in March 2007, sent to France: results negative (PCR, culture).
- No tests in 2008: no MOT,no fridge, etc.

Collars – VHF + ARGOS satellite

Figure 3 and 4. Fake collars were placed November 2006 on most of the release animals. Those not in the release group were seen using pieces of branch trying to give themselves a collar!

Collars on Males

- 2 of the males removed the collar, and it had to be put back on twice
- One male removed it again and decision not to try again as he did not keep the fake one
- *Occasion to take blood but then? No storage, nobody was leaving the release site at this time – due to the remoteness of the area, there was no way the blood could be analysed – this was an ongoing logistical issue.*

Collars on females

- Real collars were placed the day before the release
- 7 chimps in 5 hours, females put in individual transport cages

- Carried to the release site the next morning
- Good organization (the director, 2 vets, 2 keepers, 2 volunteers)
- No point to take blood few hours before release
- One female removed her collar 5 minutes before the release – was not replaced

Followup

- No close following possible (unlike the releases at HELP Congo previously) adults chimps, they hide, etc. Had to follow from distance!
- 1 chimp came back one month after release (captive born), jumped in her enclosure. Still with her new group
- 2 chimps without collar
- 1 male lost his collar in July, stays with 2 females
- 4 females+1offspring (Andrew) located, checked regularly, healthy
- 3 males followed thanks to Argos system
- Chimps will be monitored more during the dry season

- Learning from mistakes to prepare the next release! - i.e preparing from now what should be needed at the sanctuary = fridge, MOT, equipment for transport of blood, etc.
- Next release pleased planned for 2010
- Parasitology at least 2 or 3 times before release (in the 6 months before)
- Blood tests + TB tests 6 months before = time to do more investigations, to repeat tests if needed
- **Need a fridge** to keep MOT, blood samples...

5.9 ENCEPHALOMYOCARDITIS VIRUS (EMCV) OUTBREAK AT TACUGMA CHIMPANZEE SANCTUARY

R Garriga, Veterinarian, Tacugama Chimpanzee Sanctuary

Background information on EMCV

- Family: Picornaviridae; Genus: Cardiovirus
- First isolated in 1940
- Worldwide distribution
- Infects many species: mammals, birds and arthropods.
- Most times infection does not cause disease.
- Infection in humans is possible but disease is rare.
- Pathogenic in certain species:
    - Pigs are highly susceptible.
    - Non-human primates are particularly susceptible.
    - Numerous wildlife species
- Epidemiology:
    - Most probable natural hosts: rodents.
    - Virus is excreted in urine and faeces.
    - Primary route of infection is oral
    - Vertical transmission is possible
- Development of infection depends on many factors:
    - Viral dose, virus strain, species of animal, age, health, immune status (highly pathogenic in African elephants, while Asian elephants seroconvert without developing disease, never reported in gorillas)
    - Gender related susceptibility being females more **resistant** to the pathogenic effects.
- Clinical signs:
    - Sudden death is the most common sign
    - Occasional signs: lethargy, anorexia, weakness, dyspnoea, in-coordination and salivation.
- Pathological changes:
    - Pale streaked or mottled myocardium, epicardial or myocardial haemorrhage, cardiac dilatation, pale myocardium, pericardial effusion, hydrothorax, ascites, and pulmonary oedema and congestion.
- Histology:
    - Focal to diffuse, interstitial, non-suppurative myocarditis and myocardial necrosis
    - Encephalitis is rare in zoo and wildlife animals
    - Pancreatic necrosis may be seen occasionally
- Diagnosis
    - Virus isolation from heart and spleen
    - Serology: virus neutralisation tests to look for antibodies against EMCV.
    - Immuno-histochemistry from formalin tissues to confirm diagnosis.

- Treatment
  - Not available
- Prevention
  - Pest management control
  - Vaccination
- Vaccination program for susceptible species
  - Various live attenuated and inactivated EMCV vaccines have been developed but
    - The safety and efficacy in terms of maintaining sustained protective antibody titres have not been fully evaluated.
    - In many cases antibody responses were variable and short-lived.
  - An effective inactivated vaccine was developed and used in an epizootic (1993-1994) in African elephants in South Africa
  - Taronga Zoo in Australia has an history of EMCV infections causing mortality in numerous species. Annual vaccination program with an inactivated vaccine for susceptible species has been established since 2000.
  - There is currently no commercially available EMCV vaccine

EMCV AT TACUGAMA CHIMPANZEE SANCTUARY

- 12 chimpanzees died between 2005 and 2007 from unexplained causes
  - 5 cases of sudden death
  - 5 cases had mild signs of illness before dying within a week: lethargy, weakness, dyspnoea
  - 2 cases had recurrent episodes of lethargy/ weakness and died within a year

- Gender differences: 5 females to 7 males

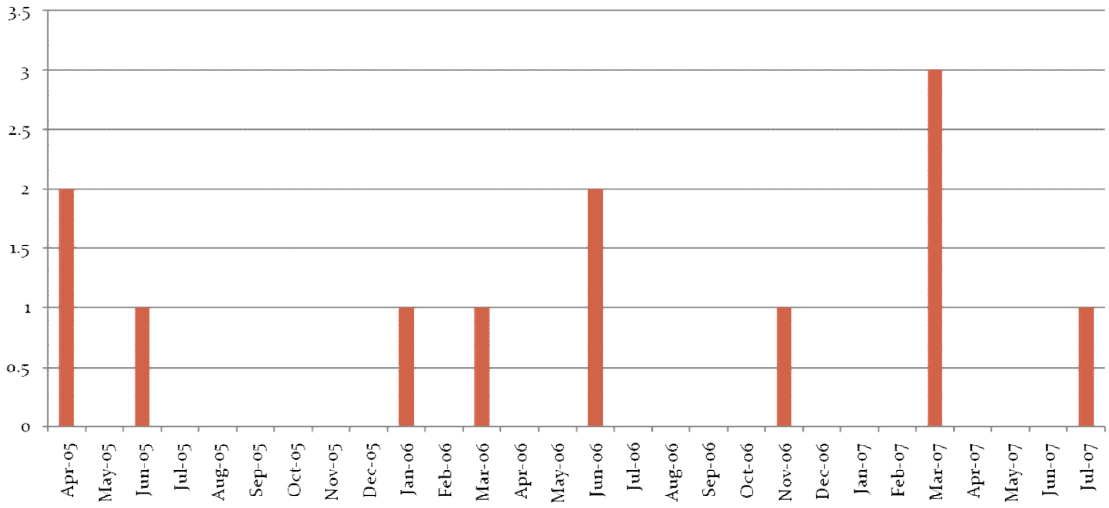
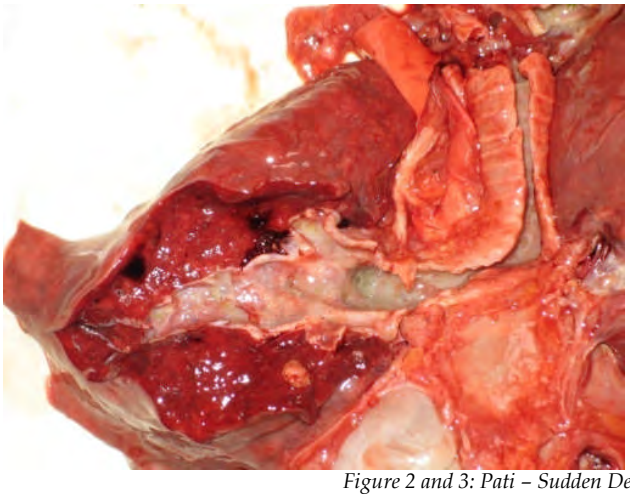


Figure 1 Cases show some seasonality – DRY SEASON

- Clinical signs:

- sudden death
- Most common signs if any: lethargy, weakness
- Sometimes: anorexia, dyspnoea, confusion, in-coordination, salivation.

- Diagnosis
  - Gross pathology (See following Figures)
    - Heart:
      - Pale streaked or mottled myocardium
      - Pale myocardium
      - Pericardial effusion,
      - Ascitis
    - Lungs:
      - Pulmonary oedema and congestion
      - Haemorrhagic lungs
    - Kidneys
      - Renal congestion



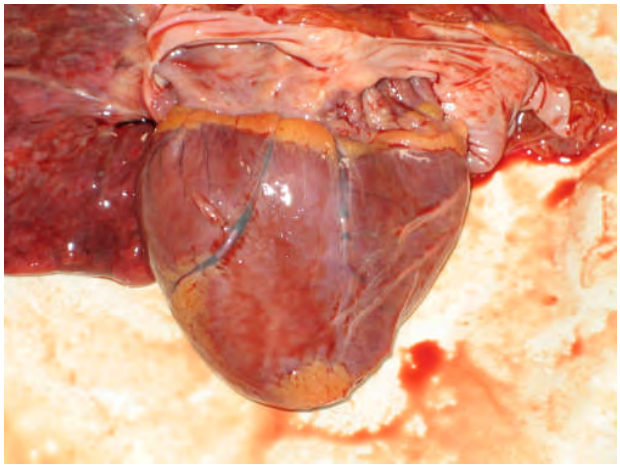


Figure 2 and 3: Pati – Sudden Death

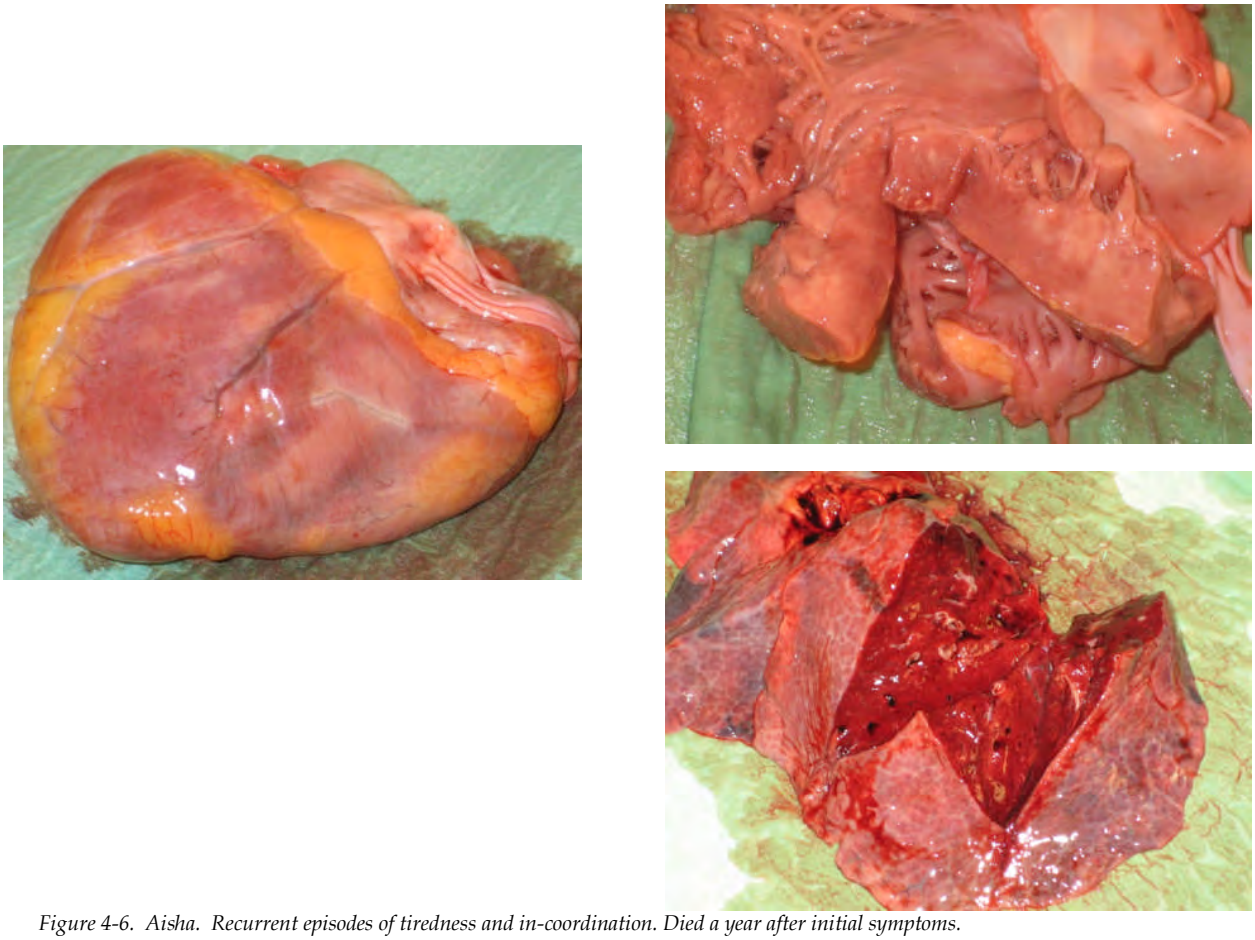


Figure 4-6. Aisha. Recurrent episodes of tiredness and in-coordination. Died a year after initial symptoms.

- Diagnosis
  - Virus isolation
    - So far the virus has only been isolated from one case.
    - Histopathological study suggests possible EMCV infection in six more cases (more results pending)

  - Histopathology (Figure 7)
    - Acute non-suppurative , necrotizing myocarditis,
    - Renal tubular necrosis,
    - bronchopneumonia

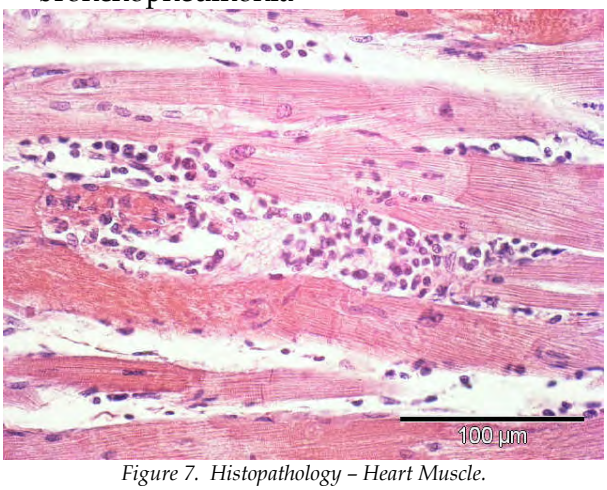


Figure 7. Histopathology – Heart Muscle.

- Retrospectively
    - Suggestive history: Clinical sings observed match the EMCV disease pattern
    - The group of chimps with more fatalities received their last meal (which consists of bulgur , beans and rice) in their den and thus leftovers remained overnight (8 cases out of 12).
    - It correlates with periods of increased rodent numbers
- Preventive measures
    - Rodent control
        - Cats, rat traps and rat poison
    - Increased hygienic measures
        - Utilise disinfectant to wash vegetables
            - Fruits are washed once, then soaked in a solution of 1% bleach, and then rinsed.
        - Food store room: rat proof and tiled to facilitated cleaning and disinfection
        - Changed feeding schedule: all feedings are done out in the enclosure to minimise leftovers in their dens during the night.
        - Feeding points in the enclosures are swept every evening to remove leftovers.
    - Vaccination
        - Using an inactivated EMCV vaccine
- Preventive measures
    - Vaccination
        - pros:
            - the disease occurs very acutely and there is no antiviral treatment available
            - A vaccine-induced immunity would substantially decrease an individual's susceptibility to the virus.
- Preventive measures
    - Vaccination
        - Cons:
            - Permits and transport of the vaccine to the site.
            - Vaccination best performed under general anaesthesia to ensure deep intramuscular injection.
            - Booster preferable at 4 weeks after initial vaccination.
            - Blood should be collected for serology to determine antibody titers at the initial vaccination and preferably again at six months to one year post-vaccination.
            - Inter-vaccination interval every 18 to 24 months
            - Cost of the vaccine and serological tests
- Importance of:
    - Comprehensive record keeping
    - Post-mortem examinations
    - Sample collection and storage

5.10 MANAGEMENT OF AN OUTBREAK OF *Mycobacteria Tuberculosis* IN A VERVET MONKEY (*Chlorocebus Pygerythrus)* REHABILITATION CENTRE IN SOUTH AFRICA

M. van Zijll Langhout and S Unwin (Vet Advisors to PASA)

Species: Vervet Monkey. Common species in South and East Africa, from Ethiopia to South Africa. Live in savannas and woodlands.

Location: Vervet Monkey Foundation, South Africa. Private sanctuary for injured and orphaned vervet monkeys since 1993. They have been a PASA member since 2006. The sanctuaries focus is on education/public awareness on the vervet monkey. Approximately 500 vervet monkeys are housed in 100 enclosures. Eleven large enclosures with 20 – 40 monkeys in each (Figure 1) and 90 small enclosures with 1 – 4 monkeys each.

Figure 1. Vervet Monkey Foundation large exhibit (Martine van Zijll Langhout)

History: In 2008, 65 monkeys died, showing 'Irritability', weight loss and heavy breathing. As the sanctuary did not have onsite veterinary cover, an external consultant was brought in. November 2008: Granulomas with yellowish, necrotic or greyish-white centres in lungs, spleen, intestines, bone and lymph nodes were found at necropsies of 3 dead monkeys. Acid fast bacilli were found by Ziehl Nielsen stain. cover the cage in a bag for gas inhalation (there are field anaesthetic kits available weighing just 6kg including oxygen) *M. tuberculosis* cultured and identified by PCR (*WARREN* et al. 2006).

Action Plan: The Department of Agriculture of the Provincial Government of Limpopo, South Africa was notified. The department inspected the centre and placed the centre under quarantine. The human health authorities were notified. An action plan to eliminate TB from the population was written.

Human Health: All staff were tested for TB (chest X-ray and ZN stain of sputum), and a new regime of testing all staff every 6 months was started. New, short-term staff are TB tested by chest X-ray prior arrival and after their stay. All previous volunteers are contacted and informed about the outbreak.

Hygiene

All contact with monkeys is prohibited. Use of gloves, facemasks and protective clothing when working with monkeys is mandatory. Strict hygiene is promoted, an information sheet about TB and talk about zoonotic disease in general is given to all staff/ volunteers at arrival. Foot baths etc are placed as needed. A new quarantine was build to isolate suspected individuals.

Non-human primate health: The centre is put under quarantine: no new arrivals or departures from the centre and no movements of monkeys within the centre are allowed. Necropsies are conducted on all dead monkeys. Each group of monkeys is tested for TB with a tuberculin skin test (Avian PPD and Bovine PPD) and the Prima TB STAT-PAK. All monkeys testing positive for the TST and/or the Prima TB STAT-PAK were removed from the group. Each group will be re-tested until the whole group tests negative 3 times.

Diagnostics – Intradermal skin testing: Tuberculin skin test (TST). Bovine PPD (left eyelid) Avian PPD (right eyelid) – Figure 2 and 3. Delayed-type IV hypersensitivity reaction. Observations at 24, 48 and 72 hours after injection. Any swelling/dropping of the eyelid was called positive (Grade 3, 4 and 5; *RICHTER* et al, 1984).

Figure 2 and 3. Avium and Bovine Tuberculin and position for intradermal injection in a vervey monkey (Photo's: M.van Zijll Langhout).

Diagnostics -Prima-TB Statpak. Full blood, serum or plasma. USDA approved lateral flow technique using selected antigens. Detects IgM and IgG antibodies against *M. tuberculosis* and *M. bovis*. Storage at room temperature. Results in 20 minutes (Figure 4 and 5). With full blood the test is sometimes not running properly. Therefore serum or plasma are recommended. Never read the test after 30 minutes.

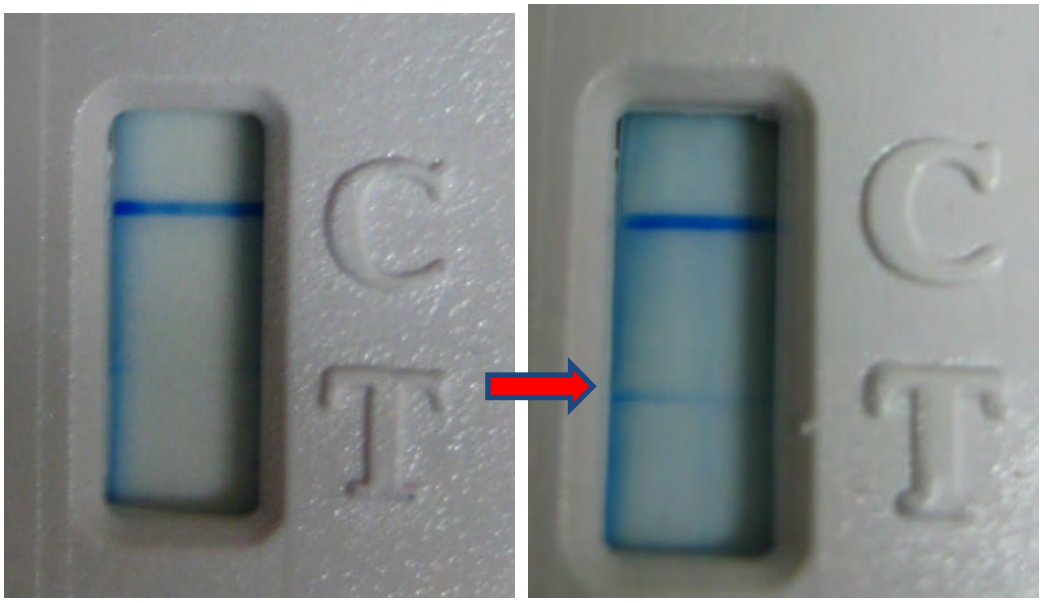


Figure 4 and 5. Prima TB statpak control and reactor (Arrow)

Testing Procedure: General anaesthetics; Ketamine IM (10 mg/kg). Clinical examination. Microchip inserted for permanent identification. Tuberculin avian PPD (right eyelid) and bovine PPD (left eyelid). Blood collection for Prima TB STAT-PAK blood assay. Blood is centrifuged and the serum is used for the Prima TB STAT-PAK testing.

Preliminary Results: 190 monkeys tested by June 2009. All 500 individuals will eventually be tested before the quarantine is lifted. Of these 190, 61 monkeys (32%) tested positive (grade 3, 4 or 5) for the TST and/or Prima TB STAT-PAK (Table 1). Most TST positive monkeys reacted to the Avian Tuberculin PPD (Table 2). In 42% of these 'TST Avian positive and TST Bovine negative' monkeys showed necrotic granulomas at necropsy and the only monkey positive for both Avian and Bovine Tuberculin PPD also showed clear macroscopic lesions (Table 4).

		TUBERCULIN SKIN TEST		
		POS	NEG	TOTAL
PRIMA TB	POS	7	15	22
	NEG	39	129	168
	TOTAL	46	144	190

Table 1: Tuberculin skin test and Prima TB STAT-PAK test results in 190 vervet monkeys.

TUBERCULIN SKIN TEST RESULTS			
AVIAN POS	BOVINE POS	AVIAN + BOVINE POS	TOTAL
40	5	1	46

Table 2: Positive reactions (Grade 3-5) to Avian and Bovine tuberculin PPD.

Necropsies: Necrotic granulomas were mainly found in the lungs, lymph nodes, liver, spleen, kidney and bone (Table 3) . Almost all individuals showed highly suspicious mesenteric lymph nodes. The mesenteric lymph nodes from each monkey and any macroscopic lesions were frozen for ZN stain and culture . Samples from lung, spleen, liver and lymph nodes were also stored in 10% buffered formalin for histopathology and PCR.

ORGANS AFFECTED IN 27 PM'S		
Organs	**Number**	**%**
Lungs	17	65%
Mes. lymph nodes	17	65%
Med. lymph nodes	9	35%
Spleen	8	30%
Liver	5	19%
Kidney	1	4%
Bone	1	4%
Other lymph nodes	2	7%

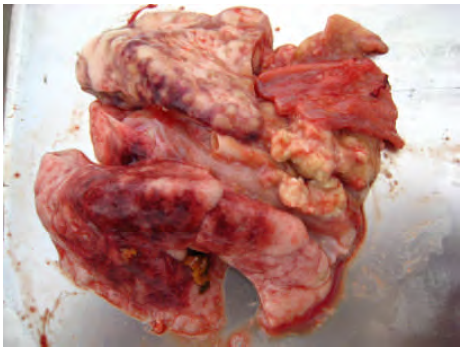
Table 3: Locations of necrotic granulomas found at necropsies of 27 TB positive tested vervet monkeys.

	NECROPSIES OF TST POSITIVE ANIMALS			
Type of TST reaction	**AVIAN POS**	**BOVINE POS**	**AVIAN + BOVINE POS**	**TOTAL**
Number of animals tested	40	5	1	46
Number of necropsies	19	2	1	22
Number animals with lesions	8	2	1	11
Total %	42%	100%	100%	50%

Table 4: Percentage of Prima TB STAT-PAK negative + TST positive monkeys showing macroscopic lesions at necropsy.

	NECROPSIES OF PRIMA TB POSITIVE ANIMALS			
Type of TST reaction	**AVIAN POS**	**BOVINE POS**	**AVIAN + BOVINE NEG**	**TOTAL**
Number of animals tested	3	4	15	22
Number of necropsies	2	2	5	9
Number animals with lesions	2	2	2	6
Total %	100%	100%	40%	67%

Table 5: Percentage of Prima TB STAT-PAK positive + TST positive and/or negative monkeys showing macroscopic lesions at necropsy.



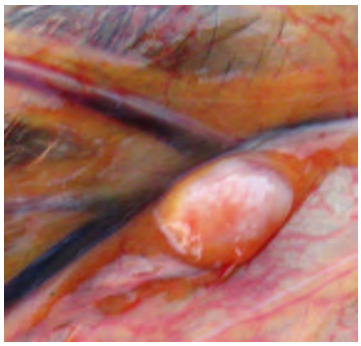
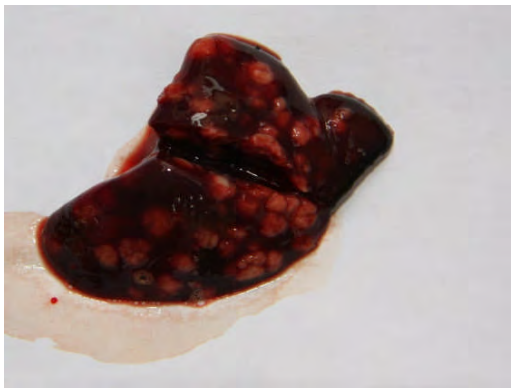


Figure 6 and 7. TB infected lung and lymph node(photos: Martine van Zijll Langhout)



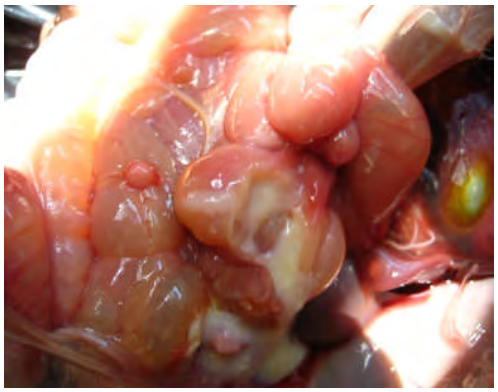


Figure 8 and 9. TB infected spleen and opened infected lymph node (Photos: Martine van Zijll Langhout)

Discussion: *M. Tuberculosis* infection has a devastating effect in non-human primate populations. The increasing frequency of both human tuberculosis and HIV/AIDS in developing countries presents a serious threat to non-human primates in rehabilitation centres in these countries. Prevention of infection by strict quarantine and human health protocols are therefore very important. Closed units and no movements of monkeys within the centre are crucial to minimize the effects of outbreaks. The testing was started in the groups where the first and most clinical cases were seen. It is expected that a lower prevalence is found in the rest of the groups. Results from cultures, histo-pathology and PCR's will give us more information about the outbreak and the sensitivity and specificity of the used diagnostic methods. Macroscopic lesions were found at necropsies in Prima TB positive and TST negative individuals and the opposite. Because the Prima TB and TST detect TB in different stages of the disease, the combination increases the chance to detect positive cases. Animals in advanced stages of TB infection may be anergic reactors in the TST and give rise to 'false negative' results. Antigens in the environment identical to the *M.avium* complex may cause 'false positive' reactions to Tuberculin avian PPD. Although the PPD's are widely used in the zoo world for TB diagnostics, there remains a need for validation of Tuberculin Avian and Bovine PPD for use in non-human primates. Necrotic abcesses may be caused by other bacterial genera (C*orynebacterium*), and so culture ('the golden standard') must always confirm TB infection. Further work is necessary to evaluate sensitivity and specificity of TST and Prima TB STAT-PAK for use in vervet monkeys. The VMF will continue to TB test all monkeys until each group tests negative 3 times. Strict guidelines, quarantine and protocols are in place to prevent outbreaks in the future.

SECTION 6: REFERENCES & FURTHER READING, including recommended texts.

This section contains all references from the text. Some of these references are also found at the conclusion of certain chapters

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