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Care for the Wild

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Post-mortem procedures
for wildlife veterinarians and field biologists
M.H. Woodford (Ed.)

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Sections I, II and III

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Preface

Since the creation of the Office International des Epizooties (OIE) in 1924, considerable progress has been achieved in the control of diseases of domestic animals; some of these diseases have even been mastered completely, or eradicated, in vast areas of the world.

The attention of the International Committee of the OIE was drawn to the epidemiological importance of diseases in wildlife in 1991. Two years later, in May 1993, a permanent Working Group on Wildlife was created, chaired by Dr M. Woodford. Since then, this Group has conducted an immense amount of work by publishing reports and guidelines and providing advice to Member Countries of the OIE on the delicate question of the surveillance and control of diseases in wildlife.

This booklet is the work of M.H. Woodford, D.F. Keet and R.G. Bengis, with a valuable contribution from the Members of the Working Group and is published jointly by the OIE with Care for the Wild and The World Conservation Union.

My sincere thanks are extended to M.H. Woodford, D.F. Keet and R.G. Bengis for sharing their expertise and to all those who have been involved in the preparation of this booklet. I would like to congratulate them on the exceptional quality of the content and presentation of this document

I am convinced that the success that this booklet will enjoy will recompense them handsomely for their efforts and will contribute much to the improvement of animal and public health across the world.

J. Blancou

Introduction

Determination of the cause of death in wild animals is often difficult and may require the close co-operation of a number of disciplines. For the diagnostic laboratory to contribute fully to final diagnosis, the specimens collected must be selected carefully and preserved in suitable conditions.

The purpose of this booklet is to assist the wildlife veterinarian, game biologist or game warden in the performance of a thorough post-mortem examination and in the correct selection, preservation and transportation of pathological and biological specimens. The reader will readily appreciate when the remarks are directed to the wildlife veterinarian or when they are for lay colleagues. Throughout this text the words 'autopsy', 'post-mortem' and 'necropsy' have been used synonymously.

The carcasses of wild animals are seldom found in suitable condition for post-mortem examination. This is particularly so in the tropics where high ambient temperatures result in very rapid putrefaction and where avian and mammalian scavengers are common.

In view of this, no opportunity should be lost to examine even a single carcass and it may be necessary to make great efforts to detect and destroy a representative sick animal. This, however, is easier said than done. Wild animals seldom exhibit the signs of disease and when they do, they often hide in thick cover. Death may be as a result of an encounter with a predator that has taken advantage of the weakened state of the sick animal.

There is usually higher mortality among young animals, especially in those species that have a high reproductive rate. Being small, the carcasses of young animals often disappear without trace.

Climatic extremes of cold, wet and drought and the nutritional stress brought about by deep snow cover or drought which curtails the growth of vegetation can alter the normal mortality rates and can also alter the population structure by acting selectively against the old, the young or pregnant/lactating females. Epizootic disease, which may be caused by an agent which is enzootic in contiguous domestic stock, may act similarly.

In the investigation of any outbreak of disease in wildlife, attention should therefore be paid to the local environmental circumstances, including those that may be affected by climate and man.

Section I

Preparing for a post-mortem examination

When mortality or morbidity in a wildlife population is reported, the investigator must collect as much general and local information as possible, including any evidence of similar mortality or morbidity in domestic stock. A thorough inspection of the surroundings of a sick or dead animal is vital. Note should be taken of signs of a struggle which may indicate an encounter with a predator or a paroxysm at the time of death. An accumulation of faeces behind the carcass may indicate a period of immobility, as may heavy browsing of food plants within easy reach. If the animal is found alive, it should be observed from a distance and note taken of any peculiarities of gait, respiration, excretion and unusual behaviour. Unusual degrees of wildness or tameness may also be important and should be recorded.

Photographs should be taken, illustrating for example environmental conditions and the appearance of sick animals and their lesions. Photographs may also be of great value if litigation is likely.

The performance of a thorough post-mortem examination plays an important part in the diagnosis of the cause of death. However, it is very important to avoid preconceptions that may limit the information and specimens collected.

In this respect it is strongly recommended that complete tissue and blood samples are collected from carcasses. If only selected samples are taken because a certain disease is suspected and the animal does not have that disease, the specimens submitted to the laboratory may not be adequate for the diagnosis of other unsuspected diseases that may be involved in an epizootic.

Notifiable diseases

If the veterinarian or field biologist suspects that the disease outbreak under investigation is likely to be caused by a legally **notifiable** disease, contact should immediately be made with the appropriate regional or national veterinary authorities before proceeding with the autopsy.

Notifiable diseases, which include **rinderpest, foot and mouth disease, classical swine fever (hog cholera), tuberculosis and Newcastle disease**, are those which are of great severity, have a high ability to spread rapidly or are of socio-economic and/or public health importance (see OIE Lists A and B on <http://www.oie.int>). These diseases are governed by strict national and international regulations.

Autopsy site

It may be possible and even desirable to transport whole, unopened small animal carcasses directly to the laboratory. Large animals usually have to be examined at the place where they are located.

If a cement floor is available (which is rarely the case), this greatly facilitates subsequent disinfection.

If possible, a site for the post-mortem remote from contact with other wild animals and domestic livestock should be selected. A pit may have to be dug to dispose of the carcass, so a site where this is feasible should be chosen. If cremation is to be the chosen method of disposal, fire risks should be taken into account.

Personal safety precautions

Before the post-mortem examination of a dead wild animal commences, it is important to consider the circumstances of the illness and death of the animal and to assess the likelihood that the cause may have been a **zoonotic** or **notifiable** disease.

Zoonoses are transmissible to humans and some can result in serious and often life-threatening infections. Extra precautions must therefore be exercised when handling and dissecting animals, which may have died of a zoonosis.

Anthrax is a common cause of sudden death in ungulates and carnivores which prey upon them. Carnivores, omnivores and insectivorous bats (and indeed almost any warm-blooded animal) may have died of **rabies** and carnivores may be infected with the small tapeworm of **Echinococcus spp.** **Tuberculosis** is an emerging disease in some wildlife populations, **brucellosis** and **Rift Valley fever** can infect wild herbivores. **Psittacosis (ornithosis)** and **Newcastle disease** in birds can cause unpleasant infections in humans. **Tularaemia, plague** and **hantavirus** are carried by rodents and/or their fleas. **Herpes B** is an important viral disease with devastating zoonotic potential but which causes minimal or undetectable morbidity in its natural wild primate host. Fruit bats and some insectivorous bats are implicated in the symptomless carriage of **Hendra virus** and **Australian bat lyssavirus** and the search for the wildlife reservoir of **Ebola virus** in Central Africa and **Nipah virus** in Malaysia continues.

In view of the above, any person conducting an autopsy on any wild animal should be aware of the risks involved and should wear appropriate protective clothing. Face masks, which cover the eyes, nose and mouth, are particularly important when examining animals suspected of infections likely to become air-borne during dissection.

Great care must be taken to ensure that all specimens taken from a carcass are collected, stored and transported safely and that there is no risk of the escape of infective material.

Protective clothing

A list of protective clothing is given below:

- rubber boots
- rubber or plastic gloves
- rubber apron

- overalls (boilable)
- face mask including goggles to cover eyes.

Post-mortem equipment

The minimum requirements for conducting a safe and satisfactory field post-mortem examination are as follows:

- a curved knife for skinning
- a straight, pointed knife for dissection
- a pair of 25 cm rat-toothed forceps
- a pair of 15 cm pointed forceps
- a pair of 15 cm dissecting scissors
- a sterile scalpel and blades
- an enterotome
- a bone saw
- a large pair of bone forceps or bone-cutting shears
- an axe
- a sharpening stone and steel
- a spring balance to weigh to 10 kg
- a block and tackle
- some nylon rope
- a small gas or alcohol burner for sterilising instruments.

The kit may be packed in a stout, heavy, wooden box.

Specimen containers and sampling equipment

The following list of equipment is necessary for sampling:

- sterile disposable 5 ml syringes and sterile needles (20 g)
- culture tubes with sterile swabs
- microscope slides in box
- sterile Universal bottles
- sterile blood tubes
- plastic bags with closure tops (Whirlpack or Ziploc type)
- heavy duty plastic sealing tape
- 300 ml wide mouthed glass or plastic jars
- a measuring tape or ruler
- rubber or plastic gloves (and talc)
- aluminium foil
- a rabies kit (World Health Organization) (or drinking straw in a small jar of buffered glycerine)
- labels, string, waterproof marker pen/pencil.

Transport equipment

For transportation, the following equipment is required:

- an insulated, plastic cooler box
- a leak-proof, screw cap, plastic containers
- absorptive packing material
- string and heavy duty plastic sealing tape
- sterile buffered 50% glycerine (see Appendix I for formulation)
- 'easy blood' (see Appendix I for formulation)
- 'blue ice' freezer packs (pre-frozen).

Fixatives

The following fixatives are used:

- 10% buffered formalin (see Appendix I for formulation)
- 100% acetone for cytology (caution! fire hazard!)
- 70% alcohol for parasites
- paradichlorobenzene.

Disinfection materials

Disinfection materials include the following:

- a plastic bucket and brush
- a nailbrush, soap and towel
- borax
- 5% formalin
- sodium hypochlorite (0.5%)
- 70% ethyl alcohol for disinfecting instruments.
- sodium carbonate (5%)

Additional equipment

The equipment listed below will also be very useful:

- field microscope (with mirror or car battery attachment for light source) for checking suspected anthrax cases before autopsy
- a portable centrifuge for serum separation
- a camera and film
- a notebook.

Section II

Post-mortem procedures

A. Ruminants

External examination

In order to minimise post-mortem changes, the examination should be carried out as soon as possible after death, especially when well insulated animals such as sheep, pigs, marine mammals and carnivores are involved. Carcasses should be weighed.

First, a thorough visual examination of the carcass should be made. All body orifices should be checked for discharges. Bloody discharges may be indicative of anthrax.

Caution must be exercised if anthrax is suspected. The carcass should neither be moved nor dissected. Anthrax bacilli only sporulate in the presence of atmospheric oxygen and there is no point allowing more spores to escape.

Two blood smears should be made on clean microscope slides with blood obtained by nicking a superficial vein, with a scalpel blade, on the external surface of the ear nearest the ground; it is often impossible to extract blood from the upper ear. If this fails, the coronary band is an alternative site. A swab can be soaked in the blood and then replaced in the sterile swab tube.

In the case of wild horses, zebras, wild pigs or carnivores suspected of being infected with anthrax, organisms may not be present in the blood so swabs and smears should be made from the cut surface of a lymph node (usually the submaxillary lymph node) or spleen, using the smallest incision.

Blood and tissue smears should be air dried and fixed in methanol. If facilities are available, the smears should be stained for two minutes in polychrome methylene blue, or Giemsa stain, washed in tapwater and examined under oil immersion microscopy. Labelled swabs should be sent by the quickest route for culture and diagnostic confirmation at the nearest bacteriological laboratory.

If anthrax is diagnosed, the carcass should be burned or buried. If possible, the carcass should be protected from scavengers while being checked for anthrax. As anthrax can infect humans, sometimes fatally, extreme caution should be exercised (Fig. 1).

If anthrax is not suspected, or has been excluded as a result of the preliminary examination and/or a consideration of the history of the case and the visual external investigation, the post-mortem examination may proceed.

The condition of the skin and pelage should be noted and an examination made for ulcers, shot holes, tooth marks and external injuries. The carcass should be turned over and any

broken limb bones recorded. The upper eye is often removed by scavenging birds and the tail

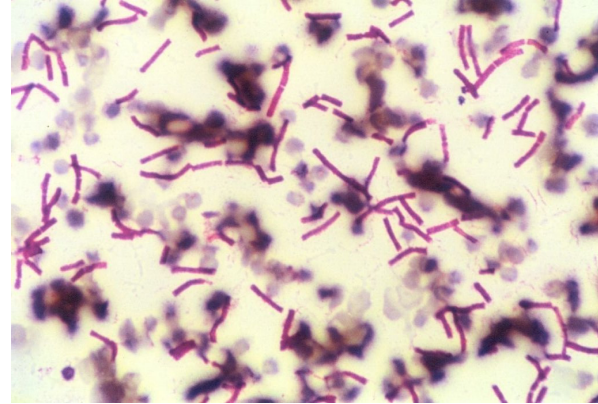


Fig. 1
Anthrax spores (IS THIS THE TITLE FOR THE PICTURE???)

and muzzle may be damaged by foxes, jackals or dogs. An examination should be made for external parasites, noting abundance and location. Representative specimens should be collected and stored in 70% alcohol. Particular attention should be paid to predilection sites, e.g. around the muzzle, eyes, ears and genitalia, on the neck, brisket, tail switch, axillae, groins and hoof clefts.

The mouth should be examined and the condition of the oral mucosa, tongue and teeth recorded. In cases of plant poisoning, parts of the plant may still be lodged between the teeth.

Post-mortem changes

Whenever possible, an estimate should be made of the time of death of the animal. Putrefaction takes place very rapidly when the ambient temperature is high. Post-mortem changes within the carcass are accelerated when the body temperature is high at the time of death, e.g. in the case of heat stroke, lightning strike, anthrax, tetanus and high ambient temperature in direct sunlight.

Post-mortem gas formation in the alimentary tract, especially in ruminants, should be distinguished from ante-mortem bloating, which itself can be a cause of death. In the latter case, there will be signs of asphyxiation.

If the carcass has been exposed to the sun for some time, the superficial musculature beneath the skin may acquire a pale, parboiled appearance ('sunburn').

When post-mortem changes are more advanced, the muscles show evidence of autolysis and

are often watery and soft. In moderate post-mortem change, the imbibition of blood and bile can be observed. In the former, haemolysed blood leaks out of the blood vessels and is absorbed into the adjacent tissues. This can readily be seen as a dark fringe along the sides of the vessels of the omentum and mesentery. Bile imbibed into the wall of the gall bladder stains the adjacent tissues yellowish green.

The condition of the heart may be used as an indicator of the time of death and degree of post-mortem change. Haemolysis of blood can produce a strong, adherent red stain on the lining of the right ventricle. The presence of unclotted blood in the left ventricle usually means that death was recent and rigor mortis has not yet set in. A disintegrating clot and dark haemolysed blood in the left ventricle is seen when an animal has been dead for 24 hours or more. This indicates that rigor mortis has occurred and that extensive post-mortem changes can be expected.

If particular interest is to be shown in changes in the brain tissues it is advisable to remove this structure first (see page ***) because post-mortem changes are rapid in the brain tissue and considerable autolysis can take place in the time required to carry out a full post-mortem examination.

Internal examination

When an antelope or other large ruminant is being examined, the carcass should be turned so that it lies on its right side. The uppermost hind leg should be raised and a cut made in the groin down to the hip joint, the leg should be laid flat on the ground. The uppermost front leg should be raised and a cut made in the axilla, extending this beneath the scapula until the leg is disarticulated. The leg is then laid flat on the ground. If the animal is a female, the udder should then be removed. Next, an incision should be made from the pubis to the base of the neck, along the midline of the belly and along the sternum. The skin is dissected back and reflected. The abdomen should now be opened along the midline, taking care not to cut into the stomach or intestines. The chest is opened by extending the abdominal incision through the costal cartilages, using a knife in the case of a young animal and a saw for an old animal.

A cut should be made just behind the last rib, at right angles to the ventral incision, right up to the backbone.

The cut edge of the rib cage should be gripped and lifted sharply upwards, breaking the ribs near the articulation with the vertebrae. In large mammals, an axe or large saw must be used to cut through the ribs at the vertebral level. The rib cage can be laid over to act as a tray upon which organs can be placed for examination. The pubis should be sawn through to expose the pelvic organs (Figs. 2, 3 and 4).

The presence of fluid in the pleural cavity and peritoneum should be noted and the amount and colour recorded. A small amount of pale yellow fluid in these two sites is normal. The lining membranes of the chest and abdominal cavities should be examined and their appearance recorded (smooth, moist and glistening or dull and granular with adhesions). The presence of

tapeworm cysts should be noted.

Fig. 2***Equines: position and incision for entry to body cavities***

The upper (left) fore- and hindlegs are reflected at their junctions with the shoulder and pelvic girdles, respectively

Fig. 3

Wild swine and carnivores: position and incisions for entry to body cavities

Fig. 4

Ruminants: position and incision to facilitate skinning

The upper (right) fore- and hindlegs are reflected at their junctions with the shoulder and pelvic girdles, respectively

Full instructions for the collection and preservation of biological and pathological specimens are given in Section II (page ***).

Next, the spleen and inguinal lymph nodes need to be examined. In the case of large ruminants, especially cervids, the rumen may have to be deflected to reveal the spleen. If the spleen appears grossly enlarged and swollen and the blood is black and tarry, anthrax can be suspected (a blood smear should be made, stained and examined **before proceeding any further with the autopsy**). If this is not possible and the animal is suspected of being infected with anthrax, the carcass should be burned or buried.

In some circumstances, when the burying or burning of anthrax-infected carcasses is difficult or impossible, due to lack of labour, equipment or fuel, the danger of fire or the sheer number of carcasses, it is possible to limit the contamination of the environment with escaping anthrax spores by ensuring that the carcass is protected by a guard for four days and nights.

The duty of the guard is to protect the carcass from avian and mammalian scavengers until the changes in pH and the putrefactive organisms within the carcass have destroyed the anthrax bacilli and prevented sporulation. After four days in the tropics the guard can be withdrawn and the carcass left to the vultures and hyaenas.

Gloves, boots and equipment should be disinfected with great care,

If the stained blood smear is negative for anthrax, the viscera can be examined.

General inflammation of the spleen and viscera, with or without abscesses in the lymph nodes, may indicate an infectious condition.

The amount of omental fat, kidney and heart fat should be recorded. The fat should be weighed after dissecting it out, if necessary.

Respiratory tract

The lungs should be examined, noting colour and consistency. The lung substance should be palpated between the finger and thumb and the presence of any firm areas recorded. The upper lung should be removed and the bronchi and bronchioles opened. An examination should be made for exudate and parasitic worms. Any hard areas should be checked by incision for parasitic lesions, hydatid cysts, cysticerci, abscesses, tubercular lesions or tumours. The trachea should be opened and checked for parasitic worms and bot fly larvae. The bronchial and mediastinal lymph nodes should be palpated and incised for tubercular lesions.

Heart, liver and spleen

The pericardium should be opened and the amount and colour of the pericardial fluid noted. The surface of the heart should be examined and any cysticerci or pale areas noted. The ventricles should be incised and the nature of the clot, if present, noted. The presence of unclotted blood usually indicates recent death and that rigor mortis has not set in (see *Post-mortem Changes*, page **). The heart valves should be examined for granular lesions and the aorta and carotid arteries for filarid worms. The myocardium should be cut in a number of places and checked for muscular cysticerci, which are also sometimes found in the coronary fat.

Next, the liver should be examined for colour and consistency, noting any cysts under the capsule. Old cysts may be calcified. The bile duct and gall bladder should be opened and examined for liver flukes (some species are very small), liver tapeworms and liver nematodes. The liver should be sliced in a number of places in order to detect any abscesses or cysts, which may be present deep in the substance.

The spleen should be examined, noting size and consistency and recording the presence of any parasitic cysts.

The omentum must be removed carefully and completely and weighed if data on fat depots are required.

The visceral peritoneum should be examined for signs of inflammation and adhesions.

Digestive tract

The entire digestive tract should now be removed, first tying off the oesophagus above the rumen and the rectum near the anus. After cutting through the rectum, a dung sample should be collected. The rumen should be opened and the consistency, colour and smell of the contents noted. If poisoning is suspected, samples should be taken from the rumen and abomasal content, together with 2 cm cubes of liver and kidney including the capsules. The specimens should be placed in clean, leak-proof jars without preservatives. Pending examination by the laboratory staff, the specimens should be refrigerated or frozen.

The rumen should be examined for rumen flukes (paramphistomes); if present, samples should be taken; the omasum and the reticulum should be opened. The reticulum should be examined for foreign bodies. The abomasum should be opened along the greater curvature using the enterotome. The condition of the abomasal epithelium should be noted and any ulcers, amphistomes and bot fly larvae (in equids) recorded. A careful check should be made for nematodes, especially red nematodes. The mucus should be scraped off the epithelium and an examination made for very small nematodes that may be embedded in the abomasal mucosa. Two ties should be made close together between the abomasal pylorus and duodenum with a cut between the ties. Similarly, the small intestine and the caecum should be tied at the ileo-caecal valve. The mesentery should be torn between the loops to stretch out

the digestive tract. Using the enterotome, the small intestines should be opened, washed and scraped into half a bucket of water. The intestinal lining should be examined for lesions and embedded parasites. The mesenteric lymph nodes should be incised and examined. The same procedure should be conducted for the caecum and the large intestine. Samples of all parasites seen should be collected and preserved. If convenient and desirable, quantitative estimations of the parasite loads, occurring in the various parts of the intestinal tracts can be made (this technique is described in Section III, page ***).

Urogenital tract

The kidneys should be examined and the capsules stripped, noting any abscesses which may be of pinhead-size in the kidney cortex; the consistency of the kidney substance should also be recorded (firm or soft and pulpy). The presence of cysts, stones and infarcts should be noted. The kidney fat should be removed and weighed if necessary. The ureters and bladder should be opened and the condition of the linings noted. Finally, the female genital tract should be opened from the vulva to the tips of the uterine cornua. The presence or absence of foetuses should be recorded as well as scars of previous implantations (in rodents and carnivores). The uterus may be inflamed and contain septic material. The ovaries should be incised and note taken of the presence of cysts, or corpora lutea. Specimens should be collected. The male reproductive tract should be examined and by palpation and the testes incised. Again, specimens should be collected.

Head

The skin and muscles should be cut at the angles of the mouth and the lower jaw disarticulated. The teeth should be examined for normal or irregular wear and the gums and alveoli for abscesses. The hyoid bones should be cut and the floor of the mouth removed, including the tongue.

The nasopharynx should be opened and checked for bot fly larvae in the retropharyngeal pouches. The tonsils and retropharyngeal lymph nodes should be incised and examined for abscesses and tuberculous lesions. The tongue epithelium should be examined for ulcers on the surface and for cysticerci and macrosarcocysts in the musculature. The parotid lymph nodes should be located and incised.

The head should be skinned and the temporal muscles removed. After severing the ears, the aural canals should be checked for spinose ear ticks (*Otobius megnini*), nymphal *Rhipicephalus* spp. ticks and ear mites. These may be embedded in masses of wax.

A saw should be used to cut through the head in front of the eyes, the sinuses and turbinate bones examined for infection, bot fly larvae (sheep and antelopes), pentastomes (carnivores) and nematodes (mustelids). The brain can be removed and examined by sawing the cranium length-wise or by carefully sawing around the cap and lifting it off. A careful examination should be made to look for evidence of haemorrhaging, bruising, nematodes, tapeworm cysts,

bot fly larvae, abscesses or inflammation of the brain tissue or meninges.

The skin should now be removed completely and the outer surface of the body of the animal examined for evidence of bruising or wounding, subcutaneous nematodes, warble fly larvae, bullet wounds and snake bites. Animals struck by lightning sometimes show evidence of burns on the subcutaneous tissues. Pale areas in the musculature may indicate 'sun burn' or 'white muscle disease' (myopathy).

The muscles may be discoloured by clostridial infections ('blackquarter'). Joints may be swollen and affected by septic arthritis.

An assessment of the general nutritive condition of an ungulate at the time of death can be made by examining the marrow in one of the femurs. The femur of a well-nourished animal will contain firm, fatty yellowish or white marrow, whereas the marrow of an animal dead of starvation or debilitated by prolonged chronic disease will be soft, watery and orange or red in colour.

B. Animals with simple stomachs

Equids

The carcass should be positioned on its left side. The left foreleg is severed by cutting all the muscular attachments that hold the leg to the chest wall. The leg is then laid over to lie flat on the ground.

The left hindleg is similarly disarticulated by cutting down to the coxo-femoral joint. An incision is now made through the skin from the anus to the chin and the body is skinned back almost to the vertebral column. The entire upper wall of the body cavity is removed by incising along the midline from the xiphoid cartilage to the pubis. From the pubis, the incision is continued almost to the tuber coxa then forward to the origin of the last rib. The ribs are now all severed near their articulations with the vertebrae using an axe or a saw.

Next, the ribs are severed along their sternal ends from the thoracic inlet to the last rib. The incision is carried back until it joins the original incision at the xiphoid cartilage.

Beginning at the rear, the severed body wall should be lifted clear of the carcass, and the underlying attachments cut, including the diaphragm. A saw should be used to cut through the pelvis, thereby exposing the pelvic organs.

The viscera are now exposed and can be examined.

In the horse tribe, particular attention should be paid to an examination of the blood supply to the great colon and caecum. This is accomplished by opening the abdominal aorta longitudinally and tracing and opening the anterior mesenteric artery and its branches. The presence of verminous thrombi, scarring and aneurysms should be noted.

The stomach, trachea and soft palate of equids should be examined for bot fly larvae.

Omnivores, carnivores and primates

Caution!

Herpes B virus infection is enzootic among Old World macaques (*Macaca* spp.), and usually causes minimal or undetectable morbidity in its natural host. Human infections are almost always fatal. A facemask should always be worn when conducting an autopsy on a primate of any species. Transmission of primate viruses from primates to humans can occur through bites, scratches, cuts and abrasions contaminated with blood or saliva, contaminated hypodermic needles and contact with infected primate tissues. Dead chimpanzees, and perhaps gorillas, may have succumbed due to Ebola virus infection and some may carry simian immunodeficiency virus.

The carcass is placed on its back. All four limbs are disarticulated and laid flat on the ground by severing the muscular attachments at the axillae and groins.

The ventral body wall is then removed from the chin to the pubis. At the sternum, the incisions pass through the costal cartilages. Care should be taken to lift the sternum while cutting the costal cartilages so that the heart is not damaged.

The ventral abdominal wall is removed completely from behind the last rib and laterally to the lateral processes of the lumbar vertebrae.

Starting with the incisions along the long axis of each mandible, the tongue, larynx, oesophagus and trachea are freed from their attachments until the thoracic inlet is reached. By holding the tongue in one hand and pulling sharply backwards, dissection continues into the thoracic cavity. The thoracic organs are removed together, attached to the oesophagus and trachea.

The spleen and omentum are then removed and the omentum weighed if necessary. The small intestines are pushed to the left to expose the rectum, which is tied off and severed. The intestines are detached and pulled forward. The loop of the duodenum is detached from the right dorsal abdominal wall. After cutting the oesophagus posterior to the diaphragm, the liver, stomach and intestines are removed together.

A small portion of the diaphragmatic muscle may be taken from carnivores and wild swine for examination for *Trichinella* spp. infection.

Caution!

When conducting post-mortem examinations on felids, canids or hyaenids, it should be remembered that they may be infected with *Echinococcus* spp. tapeworms, the microscopic eggs of which may contaminate the hairs of the tail and hindquarters. These eggs are infective for humans and develop into hydatid cysts, so rubber or plastic, disposable, surgical gloves should be worn and these should be disinfected thoroughly after use by boiling, or destroyed by burning. No known disinfectant inactivates the eggs of *E. granulosus*.

Any mammal found dead or acting strangely in an area enzootic with rabies should be considered as possibly infected by rabies and, in view of the danger of human infections, should be handled with extreme care.

In the case of carnivores suspected of rabies, the head should be severed and preserved (see Section III, Specimens for virology, page ***).

Birds

As some diseases of birds (psittacosis/ornithosis, Newcastle disease and salmonellosis) can infect humans, a face mask should be worn at all times when conducting an avian autopsy.

The carcass should be examined and the state of the plumage noted. Soiling and an unkempt appearance may indicate a long-standing illness. Pox lesions on the face and legs and mite infestation on the legs should be checked. The body should be examined for ectoparasites and the eyes and nostrils of waterfowl for flukes and leeches.

The carcass should be laid on its back and the feathers wet with water or a disinfectant solution. The carcass can be fixed on a post-mortem board with nails through the wings and feet. The skin over the ventral part of the body should be removed. The abdomen is opened by removing the ventral abdominal wall. The breast is removed by cutting forward on either side through the ribs and humero-scapular articulations. These cuts are best made with bone forceps. Examination of the upper respiratory tract is facilitated by incisions through the commissures of the beak, exposing the pharynx and larynx.

The trachea should be opened and checked, along with the eyes, for nasal leeches. The oesophagus should be opened and the entire digestive tract laid open. The presence of flukes and lice in the crop and pouch of pelicans and in the crop of cormorants should be recorded, as should nematodes in the gizzard of geese and lead shot in the proventriculus and gizzard of birds. A check should be made for large nematodes which may be seen penetrating the proventriculus musculature.

The caeca may be enlarged and hardened indicating *Histomonas* spp. infection or coccidiosis. The entire digestive tract should be examined for parasites, parasitic lesions and abscesses.

An examination should be made of the liver for white or yellow spots, which may indicate degeneration, necrosis or fibrosis. The presence of a whitish 'bloom' on the liver and other organs, often accompanied by a 'strawberry' appearance of the kidneys, indicates the presence of deposits of urates as in visceral gout.

Cheesy, mouldy masses in the air sacs and lungs may indicate infection with *Aspergillus fumigatus*.

The brain should be exposed with scissors or bone forceps. A long bone should be collected.

Other structures deserving special attention are the cloaca and tendons. A generalised inflammation of the abdominal cavity and organs may indicate 'egg peritonitis'.

Section III

The collection and field preservation of biological and pathological specimens

It is emphasised that in many cases, except when the cause of death is obvious (as when a horse is found to have suffered a fatal torsion of the large intestine), the pathologist at the laboratory carries the responsibility for the eventual diagnosis. However, the decision of the pathologist can only be as good as the specimens and data that are submitted by the field investigator.

Even when the field investigator feels competent to form an opinion regarding the identity of the disease encountered, and particularly when an exotic or unusual condition is suspected, a complete range of specimens must be taken and submitted, so that a thorough laboratory investigation can be performed.

Where possible, it is a great advantage to consult with the laboratory before embarking on the post-mortem examination so that specific instructions can be obtained on which specimens should be taken and how they should be preserved and transported.

Where possible, a live diseased animal should be submitted to the pathologist at the laboratory.

If this is impractical, as will often be the case, the next best procedure is to submit a whole, unopened carcass. However, this will obviously depend on the likelihood of the specimen arriving at the laboratory before it putrefies and becomes useless for diagnostic purposes.

More often the only reasonable action is to collect and preserve suitable samples of **all** tissues.

Specimens should be collected of all tissues that show lesions as well as those that are judged to be macroscopically normal.

Records

All specimens should be clearly numbered and labelled. The field investigator should keep a personal record, on a checklist, of all specimens taken, how they were labelled, preserved, to whom they were submitted, and by what route. All relevant information should accompany the specimens. This information may be recorded on the Post-mortem Record and Laboratory Specimen Record forms shown in Appendices II and III, respectively.

Photographs provide a useful record of the appearance of organs and lesions, and can be an important aid to diagnosis.

Preservation of specimens

The method of preservation chosen will vary with the type of investigation required and the

time that must elapse before the investigation can be made.

Refrigeration

Specimens refrigerated at approximately 4°C (38°F) will be preserved satisfactorily for a short time. Specimens for transport to the laboratory by post or other means may be packed in leak-proof containers and surrounded by ice in sealed cans or sealed plastic bags. Polystyrene boxes or large thermos flasks may also be used to provide additional insulation.

Dry ice

Solid carbon dioxide is useful but has certain disadvantages. Some pathogens, especially viruses, are partially or completely inactivated by CO₂ vapour. The specimens must therefore be completely sealed away from the refrigerant.

Delay in shipment resulting in complete evaporation of the CO₂ block may be followed by a drastic rise in temperature which could be much more detrimental to the survival of many pathogenic organisms than would be a more gradual change in temperature.

The outer container, if dry ice is used as a refrigerant, must not be airtight, as there is danger of an explosion if dry ice sublimates in an airtight box.

Most airlines have strict regulations on the transport of dry ice. These should be checked before consigning a refrigerated package.

Deep-freeze

Freezing in a deep-freeze cabinet at a temperature of -20°C is satisfactory for some but not all pathological specimens. A deep-freeze will not be appropriate for specimens destined for histology nor for such organisms as *Vibrio foetus*, *Leptospira* spp., or *Toxoplasma* spp., all of which are damaged by freezing. Serum, however, is well preserved by freezing. Whole blood should not be frozen.

Blood smears

Except when prepared for the examination of microfilaria or trypanosomes, blood smears should be thin. One should be able to read newsprint through them. In the above instances, it is of some advantage to place the thick and thin smear on the same slide. In all cases, the smears should be dried quickly, labelled with a diamond pencil or grease pencil and wrapped or boxed to prevent damage by insects. Thin smears may be fixed by dipping in methyl alcohol but thick smears should not be fixed. Blood smears are best made from venous blood collected from a live animal but useful smears can be made from blood collected from an animal which has recently died. Peripheral capillary blood may be desirable for making smears for the detection of microfilariae, which are often cyclical in their appearance in the peripheral blood (Fig. 5).

Photograph of pamphlet (black and white)
from Roy to place here

Fig. 5

WHAT IS THE LEGEND FOR THIS FIGURE?????

Thin blood smears are made as follows:

A clean slide (preferably washed in alcohol and dried in air) is placed on a horizontal surface and put a drop of fresh blood (this can be blood in anti-coagulant) near one end. A second slide, which may have its corners cut off, held at an acute angle, is placed on the horizontal slide and is drawn into the drop of blood. When the blood has spread evenly almost across the end of the spreader slide, the spreader should be pushed quickly along the length of the horizontal slide. An even pressure should be maintained on the spreader slide while making the smear. The smear is dried by waving in the air. Care should be taken to avoid exposure to flies.

Tissue smears

Tissue smears may be made for the demonstration of protozoal parasites. Fresh sections of liver, lungs or lymph nodes should be cut and dabbed lightly onto a clean slide. The smears should be dried quickly in the air and wrapped in paper or a box to exclude insects.

Blood samples (unclotted)

Small bottles containing dried ethylenediamine tetra-acetic acid (EDTA) or potassium oxalate are used to collect unclotted blood samples from animals which have just been killed. The bottle should be thoroughly but gently agitated as soon as possible after collection to distribute the anti-coagulant evenly. Vigorous shaking can cause haemolysis.

Unclotted blood samples should be stored on ice or in a refrigerator. Blood treated in this manner may be used for making blood smears or for packed cell volume (PCV) and haemoglobin estimation. If blood is collected from the cut throat of an animal, care should be taken to avoid contamination with regurgitated rumen contents that may emerge from the cut oesophagus.

It is better to collect the blood sample with a needle but if this is not possible, the skin should be cut to expose the jugular vein and carotid artery, these structures should be cut but the oesophagus on the left side of the neck should be avoided.

Blood samples for mineral analysis

Special bottles or tubes are used to collect blood samples for mineral analysis. These contain an anti-coagulant and, after mixing with the blood, are stored in a refrigerator.

Serum samples for serology

Blood for serum production should be collected as aseptically as possible in sterile glass or plastic vials. In emergencies, blood for serum separation can be collected in plastic bags or even plastic gloves.

After collection, the blood is allowed to stand for a few hours at room temperature so that it will clot. The serum is then pipetted into a sterile tube and refrigerated or deep-frozen.

If neither of these methods of preservation is available, serum can be preserved by adding 0.5% phenol or 0.01% merthiolate.

Normally, blood for serum separation is collected from the veins of a live or recently killed animal. If the collection is made with a syringe, care should be taken not to apply too high a vacuum when withdrawing the sample because this is a common cause of haemolysis, which may render the sample unsuitable for certain tests.

The red cells of some species are notoriously fragile and in these cases it is often difficult to obtain a clear serum sample. Haemolysis can also occur due to excessive agitation of the blood sample, freezing, contamination with water, over-heating (as in the glove box of a car), and bacterial contamination.

In the field, blood may have to be collected from an animal which has been dead for some time and in which the blood has already clotted. The heart is often a good source of a mixture of blood clot and serum. Such mixtures can be separated by centrifugation or sedimentation in a refrigerator. Prompt refrigeration is desirable for all blood samples taken from dead animals because bacterial contamination is certain.

Some immunological tests do not require blood serum. Examples of this are the cervico-vaginal mucus agglutination tests for vibriosis, brucellosis and trichomonosis. Mucus is collected by pipette or tampon, placed in a sterile tube and refrigerated.

As an alternative to collecting serum for serology, a minimum of 50 g of lung tissue can be collected and frozen.

Specimens for histology

Post-mortem changes occur rapidly in the tissues after death so specimens destined for histology must be fixed as soon as possible. Using a sharp sterile scalpel to avoid tearing and compression, 1 cm cubes of tissue should be cut, including a part of the capsule of the organ in the sample.

If possible, fixation should be performed at once in 10 to 20 times the tissue volume of 10% buffered formalin (Appendix I: Preservative formulations). Refrigeration for a short time is permissible but freezing or preserving with borax is not advised.

Representative samples of all tissues should be taken, including portions of spleen, liver, lungs heart muscle, kidney, brain and lymph nodes. Extra specimens of any observed lesions or abnormalities should always be included (Appendix IV). Samples can be placed in a common container of 10% buffered formalin without individual labelling.

Specimens for bacteriology or mycology

Any specimens for bacteriological examination must be taken as aseptically as possible. The instruments used should be boiled for 15 minutes before use. If facilities for boiling do not exist, contamination of instruments can be reduced by swabbing these with alcohol and then flaming until red hot. They should be cooled before use. Alternatively, if the investigating laboratory is nearby, a large specimen of tissue (>5 cm³) may be collected, placed in a clean, plastic container, refrigerated and transported rapidly to the pathologist, who will then select the specimens required for diagnostic tests.

Refrigeration is the best means of preserving tissues for bacteriology; freezing should not be used for *Vibrio foetus*, *Leptospira* spp. or *Toxoplasma* spp.

If discrete lesions are present they should be either excised, taking care to include a margin of normal tissue or an entire organ may be removed from the carcass, refrigerated and submitted.

Small animal carcasses may be submitted intact under refrigeration.

Sterile cotton or calcium alginate swabs may be used to collect samples of faeces, pus, heart blood or other body fluids. Care should be taken to see that the saturated swab does not dry out because slow drying is lethal to many organisms. Swabs may be transported under refrigeration or immersed in semi-solid bacteriological transport medium.

Specimens of heart blood, pericardial, cerebrospinal, and joint fluids should be placed in sterile bottles and stored and transported under refrigeration.

Specimens of hair and skin scrapings for mycological examination may be placed in a paper envelope or glass vial. Some animal mycoses are transmissible to humans.

Specimens for virology

Material destined for virology should be taken as aseptically as possible and transported under

refrigeration. Some viruses are very fragile and decompose rapidly after death of the host. Others are more robust and persist for a considerable time.

In the absence of refrigeration facilities, specimens for virology can be stored and submitted in 5 to 10 volumes of sterile 50% buffered glycerine solution.

In cases suspected of **rabies**, extra precautions should be taken when handling the carcass and removing the brain. For this operation a face mask and eye goggles must always be worn. If the animal concerned is relatively small, the entire head can be shipped under refrigeration in a sealed container labelled 'Rabies suspected'. Freezing is not recommended. If refrigeration is not practical, the brain can be removed from the skull and cut in half along the midline. One half should be sent in 10% buffered formalin and the other half in sterile 50% buffered glycerine solution.

Unstained smears from the hippocampus, fixed in methyl alcohol, can also be sent.

Large animals can be treated similarly, but if it is impractical to send the entire head under refrigeration, the brain may be removed and either refrigerated or preserved as above.

A safer procedure is to remove the head and insert a drinking straw through the hole in the base of the skull (foramen magnum) where it is attached to the neck. The straw should be inserted in the direction of one of the eyes. Pinch the base of the straw and hold it tightly while removing it from the brain with the sample inside it. The straw should then be cut, with the brain sample still inside it, into lengths of 1 cm and each sample, in each straw section, should be dropped either into sterile 50% buffered glycerine or 10% buffered formalin if required for histology. Although this procedure is very safe for the investigator, it only allows the pathologist to test for rabies and if the sample proves negative for rabies, no other brain tissue is then available for further tests for other diseases.

Specimens for toxicology

Generous blocks of liver and kidney, and specimens of stomach and intestinal contents should be submitted. Blood and urine may also be useful. Specimens should be placed in clean leak-proof jars without any chemical preservative. Aluminium or plastic may interfere with tests for some toxins. Refrigeration or freezing in transit is essential.

For suggested specimens for specific toxicological investigations and minimum quantities required see Appendix V.

Specimens for parasitology

Parasites are extremely common in wild animals and rarely cause clinical disease or death.

Dung samples

Faeces may be collected from the rectum of a dead animal but in this instance an attempt

should be made to estimate how long the animal has been dead. The best specimens are collected from the ground as soon as they have been passed.

A Universal bottle should be filled to the brim and faeces tightly packed down to exclude all air. The nematode eggs will not hatch in the absence of air. Samples can be refrigerated to further delay maturation of the nematode eggs if egg counts cannot be carried out promptly.

If the dung samples are to be preserved for more than a few days, a piece of cotton wool, soaked in paradichlorobenzene, may be placed in the bottom of the Universal bottle and covered with a piece of gauze. The faeces are then placed loosely on top.

If the faeces are semi-solid, holes must be pierced in the mass to allow the vapour of the paradichlorobenzene to penetrate. Specimens treated thus can be stored for several weeks but since the vapour kills the eggs they cannot be used for larval studies.

If disease due to internal parasites is suspected in a herd, dung samples should be submitted from at least five animals showing signs of disease and from at least five normal animals. The young and adolescent sectors of the population are the most likely to be affected.

External parasites

Since many external parasites tend to leave a dead animal as it begins to cool, an attempt should be made to collect some representative specimens either before or as soon after death as possible.

Ticks, fleas, lice and mites can all be collected into small tubes containing 70% ethyl alcohol. Lice and their eggs may be attached to the hairs. Mites may require a skin scraping.

A label, written in pencil, should be placed inside the tube and should note the part of the body from which the parasites were collected.

Ectoparasites can be collected from small mammals and birds by placing the entire carcass in a plastic bag containing a piece of cotton wool soaked in ether or chloroform. Stupefied parasites can easily be shaken or brushed from the body and transferred to a tube of 70% ethyl alcohol with a small paintbrush.

Ticks may be required alive if they are to be examined for infectious agents.

Not all ticks live on the host and a search should be made of nest burrows, birds nests, roosts, bat caves etc. where the hosts may spend the day or night. Soft ticks (*Argasidae*) are often found in cracks in rocks and under tree bark in such sites. These may carry infectious agents, some of which are pathogenic for humans, **so caution must be exercised to avoid being bitten.**

Live ticks should be placed in a small glass or plastic vial, the mouth of which is covered with a piece of tight weave cloth or plugged with cotton wool. A strip of blotting paper moistened with few drops of water should be placed inside the tube.

For transport, the vials should be placed in a cardboard box, loosely packed to allow ventilation. Engorged ticks travel best. The number of ticks in each tube should be limited and specimens from different animals or sites should not be placed in the same tube.

While a record of the presence or absence of ectoparasites is of some value, an account of the numbers present is much more useful. A scale such as 'less than 10; 10-30; more than 30 ticks per animal' is satisfactory. A parasite checklist should be used, always differentiating between the absence of a particular parasite and failure to look for it. In the latter case, the checklist should be marked 'N/E' (not examined).

Internal parasites

If an investigation of parasitic disease is envisaged, it is often advisable to contact the parasitologist before sending material, so as to receive special instructions concerning preservation and numbers of specimens required.

Internal parasites often break up rapidly after the death of the host and must therefore be collected as soon as possible after the host dies or is killed.

At least 50 specimens of each parasite should be collected. In the case of tapeworms, ensure that the whole worm is collected undamaged. If it is impossible to remove the head of a tapeworm or thorny-headed worm from the host tissues without damage, leave the worm attached and cut out the piece of tissue.

Worms occupying nodules should be teased out and fixed free but larval tapeworm cysts should be fixed whole.

The best parasitological material is alive and undamaged before fixing. The worms should be washed in normal (0.9%) saline solution to remove mucus and host debris, then fixed in a large volume of fixative. Specimens should **not be placed** in a bottle and then have fixative poured over them.

Nematodes (roundworms)

Specimens are dropped into hot (steaming) 70% ethyl alcohol, which kills them instantly. The dead worms should be transferred into a storage bottle containing cold 70% ethyl alcohol +2% glycerine to prevent drying out should the alcohol evaporate.

Trematodes (flukes)

Trematodes should be washed in saline and then transferred to cold 10% buffered formalin and shaken vigorously. This causes muscular fatigue in the worms and prevents them from contracting excessively. It is sometimes useful to flatten one or two flukes (not conical amphistomes from the rumen) between glass slides. The slides are held together by elastic bands and dropped into the fixative solution.

Cestodes (tapeworms)

Cestodes should be fixed in 10% buffered formalin by suspending the worm by its posterior end and dipping several times into a jar of fixative. The weight of the worm will keep it extended during fixation. A fixed tapeworm should be fully extended with all segments visible and should include the head.

Acanthocephalans (thorny-headed worms)

These worms are fixed in 10% buffered formalin ensuring that the proboscis is everted. If this is not the case, the specimen should be compressed between two glass slides until it everts its proboscis, after which the worm should be fixed.

A checklist for parasites of wild animals is given in Appendix VI.

Differential worm counts

Almost every grazing animal will be found to harbour a number of parasitic worms, irrespective of age and state of health. To assess the significance of parasitism in an investigation of field mortalities it is therefore necessary to determine not only the genera present (and sometimes the species too) but also to assess accurately the number of each genus represented. Subjective visual approximations can be very misleading, as some genera are much more obvious than others. The actual identification of the worms is best left to a specialist but the wildlife veterinarian may be able to identify them down to genus level if a microscope is available.

Collection of gastro-intestinal tract parasites for differential worm counts

Ruminants

The rumen should be opened and the ruminal epithelium examined for rumen flukes (paramphistomes). These will be found near the oesophageal inlet. Then, the abomasal/omasal junction should be tied with a piece of fine string, and the abomasum cut away from its attachments. Similarly, the duodenum should be tied off near the pylorus with two ligatures and a cut made between them. The abomasum should be opened over a bucket that has previously been calibrated with a black paint line at the 2.5 litre mark. The abomasum should be thoroughly washed, paying particular attention to the folded mucosa, and the washings added to the graduated bucket. The bucket should be filled with water to the 2.5 litre mark and mixed well but not stirred with a circular motion since this will throw the suspended worms out to the sides of the bucket. With a 50 ml beaker, 5 × 50 ml samples should be collected rapidly and placed together into a glass or plastic jar containing 20 ml 40% formaldehyde. The stoppered jar should be inverted a few times to mix the preservative with the abomasal contents. The 10% sample, suitably labelled, can now be sent to the laboratory

for specialist examination.

The small intestines are then detached from the mesentery after tying them off at the ileo-caecal valve. The intestines are opened with an enterotome over a graduated bucket, the mucosae scraped and washed and the volume made up with water to 2.5 litres. A 250 ml sample (5 × 50 ml) is taken and preserved in the same way as that of the abomasum.

Next the caecum and colon are emptied into the graduated bucket, the mucosae scraped and washed, the volume made up as before, and a 250 ml sample taken and preserved.

In the case of small ruminants, it may be possible to wash the abomasum in 400 ml of water and, with the contents included, should not exceed a total of 500 ml. Provided the total volume is made up to a suitable amount so that the 250 ml (5 × 50 ml) sample forms a reasonable fraction thereof, there is no disadvantage in preserving 50% of the abomasal (or for that matter, small intestinal) content and washings, but the percentage preserved must be clearly indicated on the label.

Animals with simple stomachs

The various parts of the digestive tract should be treated in exactly the same way as for ruminants.

In the case of the caecum and colon of equids, and indeed those of some very large animals, it may be necessary to increase the volume of water and gut content to five or even ten litres or more and to sample accordingly, bearing in mind that a 10% sample is about the smallest likely to be of value.

Labelling

Tubes should not contain too many specimens. Tubes should be filled to the brim with fixative, any air bubbles tapped out and the cap screwed on tightly. Each bottle should contain a slip of paper bearing the following information, written with a soft lead pencil or waterproof Indian ink:

- host: full common name/Latin name
- position of parasite in host: precise distribution in host
- host locality: name of area and map/GPS reference
- date of collection
- fixative used
- collector's name
- sample number.

Additional information that should accompany a batch of specimens would include a description of the clinical signs of disease, morbidity, mortality, relevant ecological data and whether the specimens represent a complete collection or just a sample.

It is a good idea to copy all this information in a letter to the laboratory under separate cover.

Acknowledgements

The author of Sections I, II and III has leaned heavily on the texts of the following publications:

1. The Wildlife Society, Inc. (1969). – Wildlife Management Techniques. The Wildlife Society, Inc., 633 pp.
2. Carter G.R. (1962). – Necropsy procedures and the submission of laboratory specimens. Animal Health Branch Monograph No. 4. Food and Agriculture Organization (FAO), Rome, 41 pp.
3. Woodford M.H. (1974). – Post-mortem procedures for wildlife veterinarians and field biologists. Project Working Document No. 4, Wildlife Management in Kenya. Food and Agriculture Organization (FAO), Rome, 40 pp.

and grateful acknowledgement is due to the authors of the above publications, their editors and publishers.

Section IV

A guide to post-mortem procedure and a review of pathological processes identified in the elephant

Introduction

This monograph is designed to provide a practical guide for an autopsy technique for the elephant, to offer hints that may facilitate this awesome procedure and to highlight the peculiarities of elephant anatomy. A standard post-mortem examination sequence is outlined, and has been adapted to the massive bulk and unique and complex anatomy of this species.

The pathological conditions described are those commonly encountered in the free ranging elephant populations in the Kruger National Park, South Africa.

Instruments

The performance of a necropsy of an elephant is a major undertaking which is physically demanding and should never be underestimated in regard to its complexity. Where possible, an individual should not attempt an elephant autopsy alone, without the necessary assistants and robust equipment.

The following equipment is recommended:

1. Four or five large butcher knives and sharpening steel or other sharpening device
2. Several robust meat hooks

3. A large saw, suitable for sectioning some of the massive bones
4. A large axe
5. A shovel
7. A block and tackle where possible
8. A wheelbarrow
9. Adequate water
10. Large PVC tray/containers and cutting boards
11. Thick ropes or chains
12. A portable gantry, where possible
13. A metal detector to locate bullets in poached animals.

In field situations, a 4x4 vehicle with ropes attached, and using a tree fork as an elevation point, may be used as a substitute for a gantry and block and tackle.

Chain saws may be useful for certain procedures, but generally this equipment tends to become obstructed with fat and sinews.

Technique

History

When approaching the dead animal, note should be made whether the animal is in sternal or lateral recumbency. Generally an elephant cannot tolerate sternal recumbency for more than fifteen minutes without expiring. This is due to its small tapering chest and large pendulous abdomen. Great caution should be exercised when approaching reportedly 'dead' elephants that are in lateral recumbency, because elephants usually sleep in this position, and at close range the autopsy candidate may wake up and attack.

The presence of vultures is frequently a good indicator of the status of the animal in the field.

External examination

The animal needs to be identified and the sex recorded. The external sexing of calves may be difficult. As a guideline, the distance between the umbilicus and the genital orifice should be measured. In females, this corresponds to approximately eight fingers in width and in males, to only four. The penis or clitoris should be exposed (in males the mucosa is a uniform blueish grey and in females it is pink). Where possible, and particularly where litigation is a possibility, the carcass should be photographed. Heavily discharging temporal glands, which are located between the orbital fossa and the external ear canal, may be indicative of severe ante-mortem stress, but is also seen in 'musth' bulls, which usually will also have urine soiling of the inner hind legs, and a rank odour.

At this stage an assessment of physical condition should also be made.

The depth of the lumbar depression and the protrusion of the adjoining dorso-lateral ridge of

the wing of the ilium affords a good overall condition-indicator (Albl, 1971). The appearance of a distinct temporal fossa and buccal depression give further indication of poor physical condition. A loose 'baggy pants' appearance of the skin over the hindquarters is also an indication of poor condition.

Collect a faecal bolus from the rectum. The presence of large, undigested portions of wood, fibre, fruit and leaves may indicate difficulty in mastication due to abnormalities of the molars, old age or abnormal diet.

Elephants may sometimes be found to have collapsed in awkward positions that may give the impression that one of the limb bones is fractured. It is important to examine that limb thoroughly because a broken leg in such large heavy animals may indeed result in death due to the lack of mobility.

All the external openings should be examined. This includes the temporal gland in the temporal depression. Excessive secretion from this gland is usually a sign of stress, which may have resulted from fear, pain or disease.

Anthrax is one of the few infectious diseases that may cause significant mortality in free-living elephant populations and may be characterised by dark, tarry, unclotted blood issuing from one or more of the natural orifices. Death is usually peracute to acute and the animal is therefore in good body condition. For this reason it is always extremely important to collect a blood smear and examine this before the carcass is skinned and opened.

Recently, encephalitis/myocarditis virus has been diagnosed as the cause of acute mortalities in free-ranging African elephants (Grobler *et al.*, 1995). In these cases, extreme cyanosis of the mucous membranes was a frequent reported finding. Although 80% of the victims were adult bulls in this outbreak, mortalities in juvenile elephants have been reported from several zoos in the United States of America (USA) (Seaman, 1987; Simpson *et al.*, 1977) and Australia.

The carcass should be examined for any penetration wounds (i.e. due to bullets or tusks). Bullets from poachers and wounds from intra-specific fighting are two of the most common causes of fatality in the elephant population studied. Most animals killed in fights are bulls, and in poached animals, the tusks are frequently removed or there are signs of attempted removal.

Skin conditions

Cutaneous papillomatosis is a fairly common benign self-limiting disease seen mainly in juvenile elephants. Characteristic warty lesions (1 cm to 6 cm in diameter), that occasionally become scarified and take on a reddish pink 'button' appearance, are found predominantly on the trunk skin, cheeks, lips and neck. They may be single or numerous, and unexpectedly are caused by a herpesvirus, rather than a papilloma virus. Recently, this virus has been shown to cause acute fatal systemic infection in Asian elephant calves in zoos in the USA, which also exhibit African elephants. The relationship of this virus to the herpesvirus causing lung nodules

(see below) is unknown.

Another condition characterised by focal raised circumscribed lesions (1 cm to 5 cm in diameter) has recently been seen in the skin of the ear pinna of young elephant. These lesions later develop necrotic centres and ulcerate. Biopsies revealed dermal capillary thrombosis with infarction, as well as perivascular lymphocyte cuffing. A viral aetiology is suspected, but no inclusion bodies have been seen.

Other skin conditions recorded are acanthotic dyskeratosis and fibrosarcoma. Severe epidermolysis of the skin, with sloughing of the lower extremities and ventral body parts, has been seen in elephants that have been burned in run-away bush fires.

Ectoparasites are generally rare on free-ranging African elephant (*Loxodonta africana*), and include the ixodid ticks, *Amblyomma tholloni* and less commonly *Rhipicephalus maculatus*. The elephant louse, *Haematomyzus elephantis* is highly species-specific, and is found in the skin folds of the head and the external ear canal. A flea, *Echidnophaga larina* has occasionally been encountered on elephants.

Skinning the animal

In the healthy animal, the skin is supple and readily moved over the underlying tissues. It is thick and in certain areas, hard excrescences or wart-like studs may be detected.

It is not always necessary to skin the entire animal, which is an arduous task, but sufficient skin should be removed to facilitate entry into the body cavities. Subcutaneous abscesses are frequently difficult to detect from the outside, because the thickness of the skin forces the abscess to dissect laterally between the skin and the muscle, and the absence of an easily detectable swelling may mask its presence.

If a perforating wound is visible, this is also a good reason to skin that specific area.

Examining superficial lymph nodes

The parotid, mandibular and superficial cervical and prescapular lymph nodes are all in approximately the same position as in other species. No popliteal lymph nodes were found in any of the carcasses examined. The thickness of the skin precludes palpation of lymph nodes in adult animals. Areas of lymphoid hyperplasia are frequently encountered in elephant lymph nodes, as are focal pyogranulomatous reactions. The documented aetiologies of pyogranulomatous lymphadenitis in free-ranging elephants are *Staphylococcus* spp., *Nocardia asteroides* and *Cryptococcus* spp.

No *Mycobacteria* lesions have been found to date in free-ranging African elephant.

Reflecting the upper fore- and hindlimb

It is not always possible to reflect the upper fore- and hindlimb in mature animals, but this can be achieved with the help of a vehicle or block and tackle, using ropes or chains.

Muscles can also be trimmed away from the bone to make the limb lighter. The limb should be separated at the coxo-femoral or scapulo-humeral joints.

Opening the abdominal cavity

The elephant has twenty to twenty one pairs of ribs with very little space between the wing of the ileum and the last rib. The incision should be a vertical one between the last rib and the tuber coxae, straight down over the bulge of the abdomen and down to the ground surface. The incision can then be extended midventrally towards the sternum.

The triangle of the abdominal wall and associated skin flap can then be lifted allowing the organs to be examined *in situ*.

With the aid of meat hooks the abdominal organs can now be dissected, loosened and removed. The abdomen normally contains 1 to 3 litres of straw-coloured fluid.

Stomach

In the adult animal, this organ is about 100 cm to 140 cm in length and about 40 cm in diameter.

The spleen lies as a long, parallel-sided strap across the left anterolateral aspect of the stomach. The cardio-oesophageal junction is clearly demarcated. The stomach then narrows towards the pylorus, lying to the right but no distinct pyloric valve is evident.

It is extremely important to study the contents of the stomach. This will reveal which plants have been ingested. In dry periods, elephants may also ingest quantities of mud in their quest for moisture. The physical appearance of the contents may also indicate a mastication problem.

Spirurid helminth parasites of the genus *Parabronema* may cause parasitic granulomas and focal ulcerations in the gastric mucosa.

The oesophagus is very short.

Remaining intestinal tract

The intestines may have a combined length of 18 m. The duodenum forms a distinct U-shaped loop and receives the openings of the bile and pancreatic ducts. The pancreas is about 50 cm long and is highly lobular. A single large duct is present which in most cases has a common opening with the bile duct into the duodenum.

A coiled jejuno-ileum then follows the duodenum.

The caecum is very large and sacculated.

The ascending colon is large and voluminous and is suspended on a mesocolon. The descending colon is relatively thick-walled and is usually easily identified by the presence of

large faecal boluses. The rectum has a powerful sphincter, which protrudes slightly on a raised projection forming the anal flap. After the abdominal organs have been removed, the diaphragm must be dissected loose from the thoracic wall.

Thoracic organs

The lungs of an elephant are large and adhere firmly to the inside of the chest wall, pericardium and part of the diaphragm by means of tough white connective tissue. There is no pleural cavity.

Thus, one option would be to proceed cranially from the diaphragm, dissecting the lungs from the thoracic wall. The other option is to chop through the ribs with an axe, loosening a large section of the thoracic wall, and then to apply blunt dissection to free the lungs.

Expansion of the lungs in the elephant appears to be mainly dependent on positive movement of the rib musculature and conical, dome-shaped diaphragm. It is for this reason that respiratory movements become compromised when elephants are in sternal recumbency, a feature that is severely compounded by pressure exerted by the voluminous abdominal organs on the diaphragm.

The trachea is about 30 cm in length and is supported by very stout cartilaginous rings, which are incomplete dorsally.

McCully and Basson (1971) reported on lymphoid nodules associated with Cowdry Type A intranuclear inclusions in epithelial and syncytial cells in the lungs of 74% of 50 free-ranging elephants that they examined. These lesions are caused by a herpesvirus and appear to be a subclinical or latent infection. Solid nodules are more frequently noticed in younger elephants and a more spongy type lesion is seen in older elephants. However, no areas of associated pneumonia were found.

Examining and opening of the pericardium and aorta

The pericardium is attached to the diaphragm posteriorly and may also be attached to the adjoining lung lobes. The pericardium consists of an outer fibrous sheath and an inner serous membrane that is reflected onto the outer surface of the great vessels. Between the serous membrane and the epicardium is a small quantity of clear yellowish pericardial fluid. The heart is large with two distinct apices. The left ventricle is approximately three to four times as thick-walled as the right ventricle.

The aorta of large bull elephants is approximately 185 cm long with a maximum lumen circumference of 20 cm (proximal end) and a minimum of 14 cm (distal end). The thoracic portion of the aorta is surrounded in its early course by a venous plexus.

Considerable individual variation in the detailed arrangement of the arterial branches occurs in the African elephant.

Spontaneous arteriosclerosis has been described. The lesions described were essentially

similar to those found in man, i.e. medial sclerosis that is characterised by the deposition of calcium in the tunica media. In advanced cases, so much dystrophic calcification has occurred that the arteries become semi-rigid pipes with minimal compliance, as the normal elastic fibres of the walls degenerate.

Elephant atheroma differ from those described in man in that they affect primarily the tunica intima. Hanks (1979) found that these cardiovascular lesions were age related and McCullagh and Lewis (1967) suggested that they resulted primarily from a repair reaction following haemodynamic damage.

An aortic aneurysm has also been described in an elderly elephant. Parasitic lesions may also occasionally be found in the vessels and muscles of the heart.

Urinary system

The urine of a healthy elephant is usually light straw coloured but often turbid with no pronounced odour. It generally has a slightly acid reaction and resembles equine urine in that calcium oxalate crystals are frequently found and the specific gravity (SG) ranges from 1.004 to 1.033.

Over 2 kg of total solids are excreted in the urine of an adult elephant daily, of which one fifth is mineral and four fifths organic matter (Benedict 1936).

The kidneys, testes and adrenals can now be removed. They are all located retroperitoneally on either side of the vertebral column in the thoraco-lumbar area. The testes hang ventral to the kidneys and are oval in shape. The kidneys are firmly held in place by the parietal peritoneum, and are slightly flattened in a dorso-ventral plane. The adrenals are elongated strap-like organs and the cortex appears dark yellow on section.

The penis can now be reflected posteriorly by dissecting it loose from the abdomen right up to the crura.

In the female, the pelvic floor can now be removed and the prominent clitoris also needs to be dissected loose right up to the crura. When the floor is removed, the female genital tract can be removed *in toto*. Placental scars are present in the endometrium of parous females, and their presence can be used to detect the number of previous pregnancies.

Other organs

The tongue and thyroid should be removed.

The large fleshy tongue is covered with filiform papillae, with sparse occurrence of fungiform papillae. There are also six circumvallate papillae on the posterior part and Mayer's foliate papillae on the margins.

The thyroid is large and bilobular and is situated ventrally to the anterior end of the trachea. Two pairs of parathyroids are usually adherent to its ventral edge.

Examining the joints

The head should be severed at the atlanto-occipital joint. The trunk can be removed at the base above and between the tusks.

To remove the brain, a horizontal section through the skull can be made just above eye level. This can be done with a chain saw or axe, after the skin and muscles of the head have been removed.

To recover the hypophysis is exceedingly difficult. This is situated in the hypophyseal fossa of the sphenoid bone and this fossa has a somewhat narrow opening through a tough fibrous membrane.

Dentition

Elephants develop six sets of molars, and these can be used for age determination (Sikes, 1968). The mandible must be removed in order to count accurately the laminae on the grinding surface of the molar, which will assist in the identification of which molar set is in use. The normal molar pattern of eruption is as follows:

| | | | | | |
|----|----------|------------|----|----------|------------|
| M1 | 1 year | 5 laminae | M2 | 2 years | 7 laminae |
| M3 | 6 years | 10 laminae | M4 | 15 years | 10 laminae |
| M5 | 28 years | 12 laminae | M6 | 47 years | 13 laminae |

Curious branched outgrowths of cement at the base of the molar sometimes occur.

The tusks can be removed with the aid of an axe from their alveolar spaces, which are found in the maxillary bone, antero-ventral to the eye socket.

Deciduous tusks or tushes are shed at about one year of age. The permanent tusks protrude beyond the lips at about 30 months and grow throughout life.

The tusks are composed almost entirely of dentine or ivory and are modified upper incisors. Initially, they have a conical cap of enamel which in later years wears off.

Usually one tusk (the master tusk) is used more than the other (the servant tusk). This master tusk generally grows thicker and heavier but frequently has the tip more blunted or worn (occasionally grooved) than the other. The pulp consists of mesenchymal connective tissue and is highly vascular containing both blood and lymph sinuses. It also contains finely branching nerves.

Defective tusks are frequently recognised by the following features (Schmidt, 1978):

- a) central black spot at the tip
- b) broken tips and longitudinal cracks
- c) irregularities of the dentine within the pulp cavity.

Black spot is caused by the deposition of branches of reactionary dentine within the enclosed

pulp canal and the open end of the canal appears at the tip of the tusk as a black spot.

Trunk

Just behind the base of the trunk on the hard palate, the orifice of Jacobson's organ can be seen.

The trunk can now be examined. It is a prehensile elongation of the upper lip and the rhinarium.

A dorsal and ventral finger-like process is present at its extremity. The nasal passages consist of two larger circular orifices separated by a fleshy septum. The canals are lined with moist epithelium. The sensory enervation of the trunk is via the maxillary branch of the fifth (trigeminal) cranial nerve, and the motor supply is via branches of the seventh (facial) cranial nerve.

More than a hundred thousand muscle motor units are involved with the fine movements of the trunk. On cut surfaces, well-demarcated longitudinal, transverse, oblique and circumferential muscle fibres are easily visible.

The head can now be sawn in half with a chain saw.

The middle ear may be opened with a hand saw or axe.

The spinal canal can also be opened to examine the spinal chord.

These last three examinations should only be attempted if specifically indicated as they are difficult and time consuming.

Detailed examination of the organs

Spleen

This is a very dark, bluish-red organ, covered by a tough whitish connective tissue capsule. Frequently, dark red, raised nodules that resemble subcapsular haemorrhages may be seen; these are normal. In a number of animals so-called 'daughter spleens' have been found attached to the stomach on the same gastro-splenic ligament.

Liver

This organ can weigh up to 68 kg in a mature male animal. It usually comprises three lobes but some individual variation may occur. There is no gallbladder and the main hepatic duct is large. Gallstones are occasionally found in the larger branches of the biliary system.

Bile duct hookworms (*Grammocephalus* spp.) are frequently found in the bile ducts and may result in biliary cirrhosis. *Dipetalonema* spp. have also been found to cause parasitic lesions in the liver.

Kidneys

There is individual variation in the lobulation of these organs and this apparently decreases throughout life, although dividing sheets of connective tissue can be located, even in the adult kidney. In the healthy wild elephant, the capsule of the kidney strips easily and is covered with large quantities of fat which is a good index of body condition.

The junction between the cortex and medulla is clearly demarcated in the healthy kidney. In some animals, the spermatic artery arises from the renal artery.

Adrenals

These paired endocrine organs are long, narrow and band-like with a lateral horn. They are located retroperitoneally and must be carefully dissected loose for further examination. On cut surfaces, the cortex is yellowish, while the medulla is grey in colour.

Heart

This organ may weigh up to 27.5 kg in mature elephants, and is unusual among mammals in that it has two apices, one on the left ventricle and the other on the right ventricle. The heart is also supplied by paired anterior venae cavae.

A useful criterion for estimating body weight is the mass of the blood-free heart. There is a linear relationship between heart weight and body mass in the ratio of 0.5 kg of heart tissue is indicative of 100 kg of body mass (Sikes, 1971).

The amount of firm, light-coloured adipose tissue in the fatty mantle surrounding the heart is indicative of health and body condition. No *Os cordis* is present, but degenerative vascular sclerotic lesions are often present in older animals. Pale areas of myocardial scarring may be detected as a result of previous infection with encephalitis/myocarditis virus.

Male genital tract

The testes are retained intra-abdominally throughout life. In adult bulls, they are large organs each weighing 1.5 kg or more, and are suspended in a retroperitoneal sac just ventral to the mid-lumbar vertebrae, partially obscuring the location of the kidneys. Consistent with this situation, the pampiniform plexus and cremaster muscles are absent. There is no distinct epididymis, this being replaced by a convoluted Wolffian duct-like structure. The seminal vesicles are large and thick walled. The bulbo urethral glands are also large and filled with a viscous secretion. The prostate is situated on the dorsal wall of the urethra, immediately posterior to the seminal vesicles.

The penis has a well-developed corpus cavernosum penis, and large paired levator penis muscles on its dorsal surface.

Female reproductive tract

A striking peculiarity is the long canal whereby the urinary and genital opening is carried to a position anterior to the hind legs, similar to that of the male. The peniform clitoris is conspicuous, lying in the ventral part of the vulva, beneath the opening of the urogenital canal. It also has levator muscles for movement, and the glans contains cartilage. The clitoris may be as long as 50 cm.

The uterus has two horns and a common body. Usually an implantation scar is permanently visible following each pregnancy because the endotheliochorial placenta is of the zonary type with deep attachments. The paired mammary glands are situated between the forelimbs, and the nipples have numerous openings.

Eyes

The iris and pupil are round and the iris is usually hazel brown in colour. The lachrymal sac duct and pore are lacking although a vestigial lachrymal gland exists. A Harderian gland opens onto the surface of the nictitating membrane, which glides transversely over the eyes by means of a special deep division of the orbicular muscle.

The dried eye lens weight can be used as an indicator of age.

Muscles

The muscles should be examined as elephants are prone to capture myopathy following severe exertion.

Blood and haemopoietic tissue

It is interesting to speculate on the sites of erythro and myelopoiesis in view of the almost total absence of red marrow in the long bone. It must be presumed that the diploë of the cranium, ribs, pelvis, sternum and vertebrae are the major sites of haemopoiesis. A *Babesia* spp. has been described in blood smears of East African elephants (Brocklesby, 1963), and various trypanosomes have also been described.

Gastro-intestinal tract

The gastro-intestinal tract should be opened and examined. Elephants are hind-gut digestors with a large caecum and ascending colon.

The parasites of the African elephant are apparently rigidly host-specific and vast numbers frequently occur in the normal animal. These include bots as well as large and small strongyles (Basson and McCully, 1971).

Additional information

An additional 'Elephant Necropsy Protocol (*Elephas maximus* and *Loxodonta africana*)' was published in January 2000 as a co-operative effort of the Elephant Species Survival Plan (SSP) Propagation Group of the American Zoo and Aquarium Association (AZA) and the Elephant Research Foundation (ERF) (American Zoo and Aquarium Association, Elephant Species Survival Plan and the Elephant Research Foundation, 2000).

Although this compilation relates mainly to the performance of a post-mortem examination on a zoo or other captive elephant, it provides a useful format for the systematic collection of information and samples that will contribute to existing knowledge of elephants in the wild and in captivity.

Conclusion

To perform an autopsy on an adult African elephant is a major operation that is time consuming and requires considerable physical effort and appropriate equipment. It is imperative to have a basic knowledge of the unique anatomy of this species to appreciate macroscopic pathological changes.

Only a few major infectious diseases have been diagnosed which may cause significant morbidity or mortality in free-ranging African elephant in Southern Africa. Of these, anthrax and encephalitis/myocarditis are the most important.

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Appendix I

Preservative formulations

- A. Sterile buffered glycerine (50%)** is used for the transport of tissue samples for culture when refrigeration is not available and is prepared as follows:
- first, mix the buffer by adding 21 g citric acid to 1,000 ml distilled water
 - in another container, add 28.4 g anhydrous sodium phosphate to 1,000 ml distilled water
 - then, mix 9.15 ml of the citric acid solution with 90.85 ml of the anhydrous sodium phosphate solution
 - finally, mix 100 ml of the buffer mixture prepared as above, with 100 ml of glycerine
 - place the buffered glycerine mixture in small Universal containers and sterilise for field use.
- B. 'Easy blood'** is used for transporting DNA from blood cells for genetic studies when refrigeration is not available and can also be used to preserve DNA for longer periods if refrigerated or frozen.

Mix 1.2 g Tris HCl, 3.7 g Na ethylenediamine tetra-acetic acid (EDTA) and 2 g sodium dodecyl sulphate (SDS) in 100 ml distilled water.

- C. 10 % neutral buffered formalin (NBF)** which is used for the fixation of tissues for histology is prepared as follows:
- to make one litre: mix 100 ml commercial formalin (38%-40% formaldehyde), with 900 ml distilled water
 - add 4 g sodium phosphate monobasic
 - add 6.5 g sodium phosphate dibasic (anhydrous).

The sodium phosphates can be pre-weighed into a vial and taken into the field in units suitable to the volume to be mixed at one time. A plastic 1 litre bottle can be filled with 900 ml of water and a line drawn to mark the level. In the field, fill the bottle with water to the mark, add 1/4 to 1/5 of the sodium phosphates and shake vigorously until dissolved. Repeat until all powder is dissolved. Then fill the bottle to the 1 litre line with concentrated formalin.

Appendix II Post-mortem record

Department or Institute: **Heading**

No. _____ Date _____

Owner _____ Address _____

Species _____ Sex _____ Age _____ Weight _____

Common and Latin names _____

Laboratory specimens submitted _____

(See attached checklist) _____

History _____

Preliminary examination: time from death to autopsy _____ hours

Condition of carcass (1) Post-mortem change _____

(2) Nutritional state _____

Description of examination of tissues

1. Oral and thoracic _____

2. Parenchymatous organs (liver, gall bladder and bile ducts, kidney and ureters, adrenals, spleen and pancreas) _____

3. Digestive organs _____

4. Urogenital organs _____

5. Skeletal _____

6. Head _____

7. Brain and cord _____

8. Parasites _____

(see parasite checklist) _____

Appendix III Laboratory specimen record

Department or Institute: **Heading**

Identification No. _____ Disease suspected _____

Owner _____ Examined for _____

Specimen _____ Address _____

Preserved by _____
(formalin, freezing, etc.) _____

Forwarded to laboratory, **date** _____ a) by post
b) by rail
c) by air
d) other specify _____

Species _____ Sex _____ Age _____ Weight _____

Common and Latin names _____

Clinical history, symptoms _____
(clinical tests, etc.) _____

Post-mortem notes _____

Separate post-mortem form included _____

Date conducted _____ Hours after death _____ hours

Name of the veterinarian _____

Address _____

Appendix IV

Checklist for fixed tissues

All tissues listed below should be preserved in 10% buffered formalin (one part by volume of tissue to 10 parts by volume of fixative). Tissue samples should not be thicker than 0.5 cm. A sample of **all** lesions seen and of **all** listed tissues should be included.

Tissues

- Salivary glands
- Oral and pharyngeal mucosae
- Tonsil
- Tongue (cut across tip)
- Trachea
- Lungs (specimens from several lobes)
- Thyroid and parathyroid glands
- All major lymph nodes (cervical, mediastinal, bronchial, lumbar, mesenteric etc. sectioned to 1 cm³)
- Thymus.

Heart (sections from auricles and ventricles and from valves)

Liver (three representative specimens, including bile duct and gall bladder)

Spleen (representative cross-section including capsule)

Oesophagus (representative section: 3 cm)

Stomach (several specimens from all areas)

Intestines (3 cm representative specimens from each region)

Omentum (specimen of 3 cm)

Adrenal glands (whole gland cut in half)

Kidney (1 cm³ from cortex and medulla of each kidney)

Urinary bladder (ureters and urethra, cross-section of bladder, including mucosa; 2 cm sections of ureters and urethra)

Uterus and ovaries: if possible the complete uterus (plus contents) should be preserved with ovaries attached. Uterine horns should be incised to allow entry of fixative. If this results in a specimen which is too bulky, representative samples of the cervix and uterine wall should be preserved. Ovaries should be preserved whole or, if large, should be cut transversely.

Testes (0.5 cm³ section of each testis, including capsule)

Epididymis (representative sample)

Prostate gland (whole gland or, if large, representative 1 cm³)

Eyes (whole eye, with sclera incised to allow entry of fixative)

Brain (cut in half, with one half preserved in buffered 10% formalin and the other half preserved for virology and toxicology, see Section III, page ***)

Spinal cord (sections from cervical, thoracic and lumbar regions)

Diaphragm and skeletal muscles (representative samples of major muscle groups)

Bones (sawn section of femur including marrow)

Skin (section of abdominal skin, lip and ear pinna)

Neonates (umbilical stump and surrounding tissues should be preserved).

Appendix V
Suggested specimens and minimum quantities to submit for toxicological examinations

| Type of poison | Specimen for analysis | Minimal amount required (or send all available) |
|------------------------------|--|---|
| Alkaloids | Blood, urine | 200 ml |
| | Liver, stomach content, kidney, brain | 500 g |
| All poisons | Bait or feed | 500 g |
| Barbiturates | Blood | 200 ml |
| | Brain, fat | 500 g |
| Chlorinated hydrocarbons | Fat, liver, brain | 500 g |
| Chronic arsenic | Skin, hair, stomach contents, liver, kidney | 500 g |
| Chronic lead | Bone | 500 g |
| Fluoride | Bones, teeth | 200 ml |
| | Urine, blood | 200 ml |
| Heavy metals | Urine, blood | 200 ml |
| | Stomach contents, liver, kidney | 500 g |
| Hydrocyanic acid | Blood, urine | 200 ml |
| | Stomach contents, liver, muscle (freeze immediately) | 500 g |
| Mycotoxins (e.g. aflatoxins) | Blood | 200 ml |
| | Stomach contents, liver, kidney | 500 g |
| Nitrate, nitrite | Blood | 200 ml |
| | Stomach and gut contents (sealed in airtight containers) | 500 g |
| Organo-arsenicals | Hair, liver, kidney, stomach contents | 500 g |
| Organophosphates | Blood (for cholinesterase) | 200 ml |
| | Brain, fat | 500 g |
| Oxalates | Urine | 200 ml |
| | Kidney, stomach contents | 500 g |

Appendix V (contd)

| Type of poison | Specimen for analysis | Minimal amount required (or send all available) |
|-----------------|---------------------------------|---|
| Selenium | Blood | 200 ml |
| | Liver, kidney, hair | 500 g |
| Sodium chloride | Stomach contents | 500 g |
| Strychnine | Urine | 200 ml |
| | Stomach contents, liver, kidney | 500 g |
| Sulfonamides | Kidney | 500 g |
| | Urine | 200 ml |
| Thallium | Urine | 200 ml |
| | Kidney, stomach contents | 500 g |
| Warfarin | Stomach contents, liver | 500 g |
| | Blood | 200 ml |
| 1080 | Stomach contents | 500 g |

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