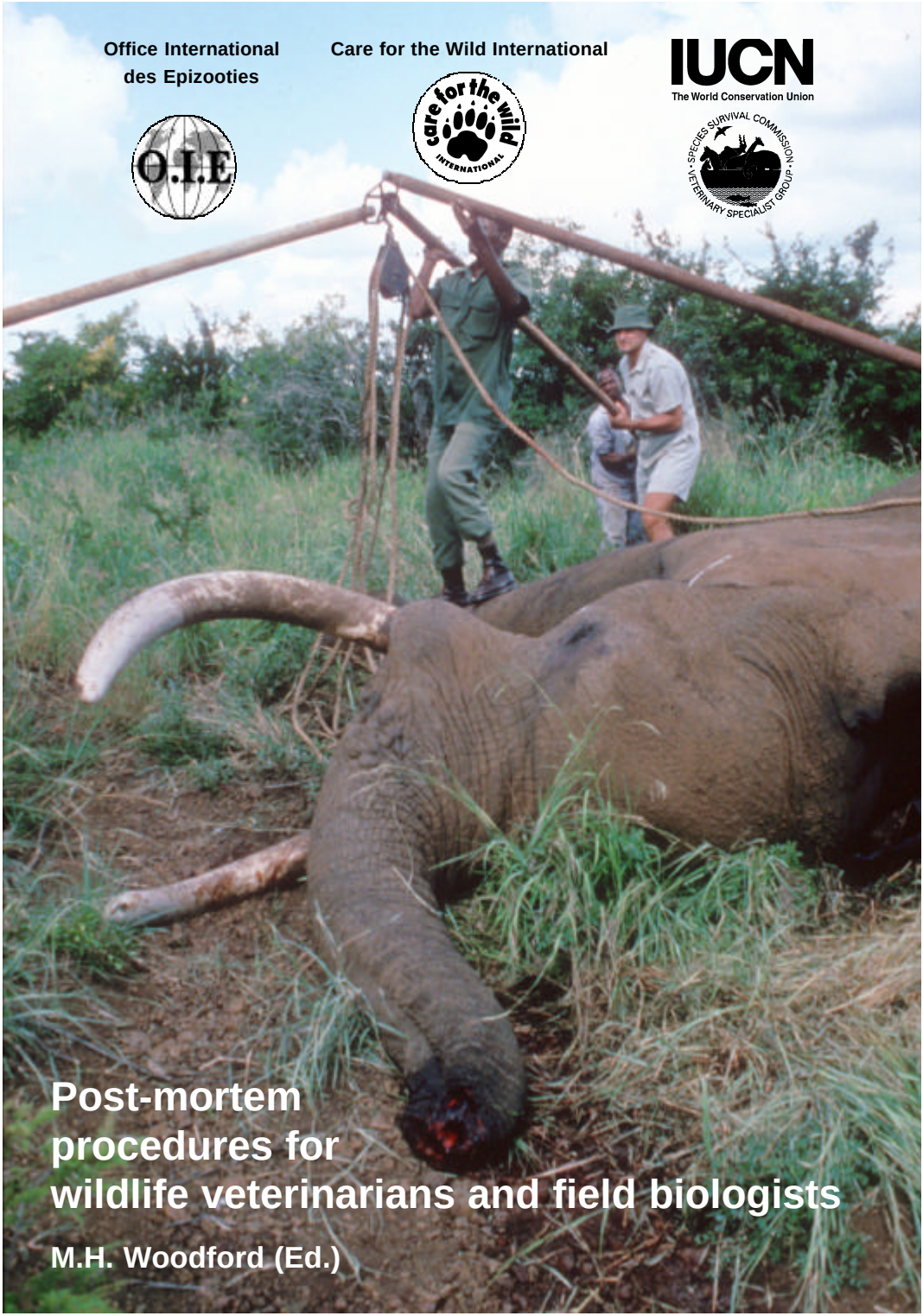

Post-mortem procedures for wildlife veterinarians and field biologists

M.H. Woodford (Ed.)

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Post-mortem procedures for wildlife veterinarians and field biologists

M.H. Woodford, D.F. Keet & R.G. Bengis

(M.H. Woodford, ed.)

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Section IV
A guide to post-mortem procedure and a review of pathological processes identified in the elephant
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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-mortal 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 pyloris, 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 retroperitonealy 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 maxilliary 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, the ratio being 0.5 kg of heart tissue corresponding to 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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