BASIC POSTMORTEM REQUISITES FOR ZOO VETERINARIANS

केन्द्रीय चिड़ियाघर प्राधिकरण

Central Zoo Authority

(STATUTORY BODY UNDER THE MINISTRY OF ENVIRONMENT & FORESTS, GOVT. OF INDIA)

THE BASIC POST-MORTEM REQUISITES FOR ZOO VETERINARIANS

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FOREWORD

The value of wildlife has been widely ignored or under-rated in the past by the international community. However, the scenario is changing and there is an increased awareness among general public towards wildlife conservation and its management. The Central Zoo authority of India has taken up the onerous responsibility in this connection as a wildlife custodian, with the help and support of wildlife scientists and veterinarians. The task is very important and has an ultimate relevance to human welfare.

The veterinary profession though largely livestock oriented, of late is considering the prospects of wild animal production. The importance of wildlife to local community has also be recognized globally. Considering the importance of diseases in wildlife conservation and management, the authors have prepared a guide for the use of field wildlife veterinarians and para-veterinary wildlife staff on issues of post-mortem and disease investigation relevant to wildlife. The authors deserve special appreciation for their meticulous efforts in providing a comprehensive picture of wildlife pathology, which is an increasingly subject of importance. Gross post-mortem examination and laboratory investigation of samples provides an opportunity for early diagnosis, for detection of new or emerging conditions and for research on the pathogenesis and epizootiology of infectious diseases.

This publication ***"Basic Postmortem Requisites for Zoo Veterinarians"*** *has been provided by experts from various fields and rightly funded by the Central Zoo Authority of India, a statutory body of the Ministry of Environment and Forests, Government of India.*

The Chapters in the book address the approach and rationale to gross post-mortem examination, the principles of sampling and transportation of specimens, the laboratory tests required depending upon the circumstances and the possible diagnosis.

Our knowledge of wildlife diseases and the diagnostic techniques are rather limited and the challenges are many. As more and more information is gathered and with the advent of technology there will be a need for continued revision of the existing publication. This will be a handy and useful guide book for zoo veterinarians for proper preservation and submission of samples for investigation.

I congratulate the authors for their efforts which reminds of the importance of sound pathological techniques to clinical diagnosis.

Place : Chennai – 600 051
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(R.KADIRVEL)
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CONTENTS

INTRODUCTION

The different species of wild animals survive and maintain a balanced ecosystem, inspite of periodic disease outbreaks and epidemics. Constriction of their habitat results in increased density, thus precipitating the occurence of disease. Further, wildlife being virgin population, and without any periodic vaccination for various diseases - are all the more susceptible ones for many diseases. Transmission of diseases may occur between domestic animals and wild animals as well. However, we do not have sufficient records of disease occurence and maping of various disease zones in our wild animal population, which are our nation's valuable resources.

This may be due to

1. Absence of scientific documentation of morbidity and mortality records and laboratory confirmation of disease.
2. Deficiency in training and the number of trained wildlife veterinarians, as most of the veterinary colleges do not have wildlife health and disease management in the curriculum.
3. Difficulty in locating a qualified veterinarian to conduct postmortem examination.
4. Difficulty in transporting the dead to the nearest veterinary institution.
5. Difficulty in getting access to a dead wild animal before putrefaction for conducting postmortem examination.
6. Mutilation or loss of carcasses by scavenger animals.

Particulars pertaining to morbidity and mortality, occurence of epidemics and disease in wildlife as well as in domestic animals are essential to monitor and assess risks due to disease in both domestic and wild animals. Because most disease outbreaks in wild animals are transitory in nature and short lived; Each reported occurrence of disease, must be treated as a matter of urgency and as much information as possible must be collected. More over, disease outbreak in wild animals resulting in high mortality may also attract and excite public and media attention.

The veterinarian is often expected to find out the cause and to suggest and institute the control measures. It becomes a tough task, as it is often difficult to identify an intact, affected animal for the assessment of diseases, particularly if it is going to be an insidious disease. The veterinarian must visit the site of the outbreak; because there is no substitute for direct examination. Veterinarian must go fully equipped to perform postmortem examination, collect data and a variety of specimens. The field para-veterinary personnel may also be trained to assist during the postmortem examination by a qualified veterinarian, and to collect samples from dead wild animals.

The preparation and despatch of these samples for laboratory investigation can also be taught to the field staff so that they can assist the veterinarians while conducting postmortem examination.

I. SPECIALIZED ANATOMICL FEATURES IN WILD ANIMALS AS BASIS FOR POSTMORTEM

1. Non-human primates

a. General Features

Cheek pouches are present in macaques.

Nipples (paired) are in brisket (pectoral position), as in case of homosapiens or elephants.

Scrotum - external

Tail is present in many species but is absent in species like:

- Gibbons, Loris, Chimpanzee

In general, the arms equal the length of legs.

Highly lengthened hands are present in :

a. Gibbons (eg. Hyalobates hoolock)

b. Orangutans

In certain species, legs are highly prolonged. (eg. Sifako monkeys) Primates have 5 fingers in hand and 5 digits in limbs.

Note

The first finger and first toe are more or less opposable; finger are capable of spreading and grasping.

b. Chimpanzee

More furrows and convolutions in brain, like man.

c. Bonnet Macaque

Bonnet like shield in eyebrow is present.

Tail is longer than rhesus macaque.

Looks similar to rhesus macaque.

Sex skin is present in females.

d. Rhesus Macaque

Tail is shorter than bonnet macaque (it is a differential feature)

Sex skin is present in both males and females.

e. Slender Loris

Very long slender arms and legs.

Large eyes and large ears are characteristic.

f. Aye-aye

Middle finger is so long which serves to dig food out of narrow cavities.

g. Tarsier Monkeys

Suction cups are present at the tips of finger and toes.

h. Lemur, Loris & Bushbaby

- Broad palm
- Broad sole of foot

- Flattened fingers
- Sensitive pads & balls of fingers, as seat of refined sense of touch

i. Brown Capuchin

- Fully furred tail
- Erect hairs on head forming horns (or) skull cap

2. Elephants *(Elephas maximus)*

1. In adult elephant, pleural space is absent and the lungs adhere to the chest wall by fibrous connective tissues.

2. Gall bladder is absent.

3. Dental formula for elephants:

I	$\frac{1-1}{0-0}$	1st molar is shed at 2nd year
C	$\frac{0-0}{0-0}$	2nd molar is shed at 6th year
P	$\frac{3-3}{3-3}$	3rd molar is shed at 9th year
M	$\frac{3-3}{3-3}$	4th molar is shed at 20th-25th year 5th molar is shed at 43rd year 6th molar is shed at 60th year

4. Musth is the physiological state in male elephants, during which time, enlargement of temporal glands near cheek region is noticed.

5. Testicles are intra-abdominal.

6. Tusks are the modified upper incisors present in male elephants.

7. Tushes (small under developed tusks) are the structures seen in female Asiatic elephants.

8. Mammary glands are in pectoral region (1 pair).

9. Life span is 60-90 years.

10. Paired venacava and bifurcated apex are the unusual cardiac structures present in the elephant.

11. Pharyngeal pouch is present.

12. Vagina is vertical and lengthy.

13. No bones are present in the trunk.

14. Anal flap is a specialized structure near the anal region.

15. Caecum is large.

16. Kidney - surface is lobulated, as in bears.

3. Bovids

Like domestic bovids, the species like gaur or wild buffalo have common features.

1. Compound stomach is present.

2. Lateral toes are rudimentary, called the dewclaws.

4. Equids

1. Gall bladder is absent.
2. Caecum is large.
3. In general, the anatomical features are similar to that of domestic horses.

4. Digital cushion is present near the foot-pad.

5. Camelids

1. Camelids are pseudo-ruminants.

   (The gastric region of camelids differ from that of the ruminants and the stomach has three compartments; Saccules are present in the first compartment and second compartment is lined with glandular epithelium).

2. Jugular vein is superficial only for a short, 8 cms from its origin until the vein passes between the muscles of the neck to meet the carotid artery and vaso-sympathetic trunk.

3. Anatomy of camelid foot is also unique in nature and the foot has 2 digits and the plantar surface is enriched with a soft-cornified epithelium similar to that of the bulb of the heel in sheep or goats.

4. Beneath the protective epithelium of the foot, fatty fibroplastic pad is present and this is similar to the digital cushion of the horse.

5. Dental formula of Ilama:

$$I\ \frac{1}{3}$$

$$C\ \frac{1}{1}$$

$$PM\ \frac{1\text{-}2}{1\text{-}2}$$

$$M\ \frac{3}{3}$$

6. Dental formula of camels

$$I \ \frac{1}{3}$$

$$C \ \frac{1}{1}$$

$$PM \ \frac{3}{2}$$

$$M \ \frac{3}{3}$$

7. The penis has a sigmoid flexure, similar to that of other artiodactylids and the prepuce is directed backwards in the unaroused male.

8. No seminal vesicles are present.

9. Gall bladder is absent.

10. Soft-palate has a structure (pouch) called as "gula".

6. Rhinoceros

1. Gall bladder is absent.

2. The caecum is very large and has a simple stomach.

3. Kidneys resemble almost like those in bovids but the lobulations are more deep.

4. Indian rhinoceros has a single horn, whereas the African species has got two horns which are usually much lengthier than the horn observed in Indian rhinoceros.

5. Dental formula :

$$I \ \frac{0\text{-}1}{0\text{-}1}$$

$$C \ \frac{0}{0\text{-}1}$$

$$PM \ \frac{3\text{-}4}{3\text{-}4}$$

$$M \ \frac{3}{3}$$

6. Horns have no real skeletal support but are composed of keratinized cells growing from the epidermis, covering a cluster of long dermal papillae.

7. It has armor like hide and three-toed feet.

8. Horny pads are present in the feet and has short-legs.

7. Hippopotamus

1. Skin is smooth which is almost hairless, except at the tips of the tail and ears and there is also sparse growth around the lips.

2. The dental formula is:

$$I\ \frac{2\text{-}3}{1\text{-}2}$$

$$C\ \frac{1}{1}$$

$$PM\ \frac{4}{4}$$

$$M\ \frac{3}{3}$$

The canine teeth is elongated into tusks that are generally covered by lips.

3. Complex stomach with four distinct sections are present but however these species are not ruminants.

4. Caecum is absent.

5. Gall bladder is absent.

6. Kidneys are lobulated.

7. Nile hippo has reddish secretion which is a thick and oily product of modified sebaceous glands, serving to protect the skin from both water and desiccation.

8. Membrane - i.e. web is present attaching the four toes together and this is one of the unique features among the artiodactylids.

8. Cervids

1. Velvet is the term employed to thick soft skin enhancing the grown antlers and is highly vascular, highly fragile and is noticed in chital, hog deer, sambar, manipuri deer etc.

2. Antlers are present in all male deers except the followings:
   - Males of chinese water deer
   - Males of musk deer

The reindeer or caribou is the only species in which the female normally has antlers.

3. All members of cervids have no gall bladder except the musk deer.

4. Canine teeth are absent in all deers except in the case of musk deer and chinese water deer.

5. The lateral digits are rudimentary on each foot.

6. Most cervids have preorbital glands and the inter-digital glands.

7. There are 4 nipples on the mammary gland.

8. The general dental formula of cervids is:

$$I\ \frac{0}{3}$$

$$C\ \frac{0}{1}$$

$$PM\ \frac{3}{3}$$

$$M\ \frac{3}{3}$$

9. Deers have compound stomach since they are ruminants.

9. Giraffe

1. The upper lip overlaps the hairy lower lip.

2. The bony protuberances (sometimes called as horns) on head are permanent, unbranched and skin covered.

3. Gall bladder is absent.

4. Four mammary nipples are present.

10. Pronghorn

1. Scent gland is present (called as the croup gland) at the end of the sacrum; Hence, the name 'blink-stink' antelope.

2. Gall bladder is present.

3. No dewclaws on the pronghorn.

4. Females have four nipples.

11. Tapirs

1. Digestive system resembles that of horse.

2. Dental formula:

$$I \quad \frac{3}{3}$$

$$C \quad \frac{1}{1}$$

$$PM \quad \frac{4}{3\text{-}4}$$

$$M \quad \frac{3}{3}$$

3. The nose and upper lip are extended to form a short but highly mobile trunk.

12. Felids

1. Presence of retractable claw is a characteristic feature in felids; All felids (except cheetah) have their retractable claws that are covered by an epithelial envelope when retracted.

2. An elastic cartilaginous band replaces the bony intermediate protuberance characteristic of the hyoid structure and larynx is one of the anatomical characteristic features.

3. Rugae are present in stomach.

4. Prominent and horizontally placed folds are present in oesophagus.

13. Suids

Wild suids are similar to domestic suids, in general.

1. Pigs have two-chambered, non-ruminating stomach.

2. Dental formula is:

$$I \ \frac{3}{3}$$

$$C \ \frac{1}{1}$$

PM $\frac{4}{4}$

M $\frac{3}{3}$

3. The upper canine teeth turn upward and form the tusk.

4. Thick layer of subcutaneous adipose tissue is one of the characteristic of these animals.

14. Pangolins

1. Eyes are small and get surrounded by thick lids that provide protection against the bites of ants and termites.

2. Lengthy tongue is present (it is about 16-40 cm in length) and tongue when unused, is kept inside a sheath located in the chest cavity.

3. The salivary glands extend almost to the shoulder.

4. Teeth are absent.

5. The lower jaw bones are represented by flimsy rods of bone.

6. Stomach of pangolins is muscular (it's function is similar to the avian-gizzard) and has horny, laminated epithelium.

7. The central three front claws are much enlarged. (These are used to dig and break open the ant and termite nests).

8. Large, bean-shaped glands that has a noxious fluid (used for defense purpose) are present in clusters around the anal region.

9. Encountering of sand and pebbles in the capacious stomach that functions similar to the avian-gizzard shall not be an abnormal feature in case of pangolins because certain quantities of sand and pebbles are ingested along with the insects.

15. Edentata

1. Elongated snot with tubular mouth is a specialised feature in anteaters.

2. Teeth are absent in ant eaters.

3. Long tongue (which is sticky with saliva during feeding) is another specialised antomical structure in anteaters

4. The testicles of the males are located within the abdominal cavity.

5. Double posterior venacavae and a retemirabile in each extremity (which functions as a counter-current heat exchanger) are present.

6. The female edentates have a cloaca with a common urinary and genital duct.

16. Marsupials

1. Webbed feet with short, thick, fur-covered paddle like tail is present in platypus.

2. Pouch (or) marsupium is present in marsupials and this is one of the major distinguishing feature of marsupials when compared to the mammals;

Pouch is present only in females and some species develop the pouch only while suckling, as the case with echidna.

3. In female marsupials, the two lateral vaginas become united anterior to the ureters, where a median vagina is formed.

4. Between the median vagina and the urogenital sinus, a birth canal is formed in the connective tissue and through this birth canal only, the fetus is ejected.

Note :

In most marsupials, the birth canal is temporary and gets reformed at each birth but in most Kangaroos and wallabies, it becomes lined with epithelium and remains patent after the first birth.

5. Like Monotremes, a pair of epipubic bones articulating with pubis is possessed by marsupials and these are not found in any eutherian mammal.

6. Syndactyl claws (first digit on hind foot is greatly reduced and the second and third digits are partially fused and reduced in size; This condition is called as "syndactylism") are present in animals belonging to Macropodidae, Vombatidae, Burremyidae, Tarsipedidae, Peteuridae and Phalangeridae.

17. Aves

- Salivary glands are absent in water birds and are well developed in birds that eat dry feed items.

- Unlike mammals, oesophagus is longer and has larger diameter also.

- In psittacine group of birds, intrinsic muscles are present in tongue.

- Humming birds have tubular tongue.

Crop

Crop (dilated part of oesophagus just cranial to the thoracic inlet)

Note

Crop is absent in certain aviary species

eg. Owls, Gulls, Penguins

Crop does not have any mucous glands, unlike oesophagus.

Sustainable diverticula

Male birds of some species have inflatable oesophageal diverticula which get inflated with air during sexual displays:

eg. Pigeon, Ostrich, Sand grouse, Great bustards

- Proventriculus (glandular stomach) and ventricular stomach (muscular stomach or gizzard) are present.
- Isthmus is the intermediate zone between proventriculus ventriculus.
- Liver has 2 lobes (right lobe is longer than left and other lobe is frequently subdivided).
- Gall Bladders:

  This is absent in following species:

  Psittacines, Columbiformes, Ostriches

However, gall bladder is present in many species of birds.

- Meckel's (vitelline) diverticulum is present, arbitrarily between jejunum and ileum.
- Pancreases is present in between duodenal loop.

- Caeca :
  - This is absent in budgerigars.
  - Caeca is rudimentary in other psittacines.
  - Often in general, paired caeca is present in birds.
  - Caeca are present in followings:

    Gallinaceous species, Water fowl, Owl, Ratities

Note:

Even among ratites, the ostriches have large sacculated caeca, while the emus, cassowaries and rheas have only vestigial caeca.

- Birds have wing and collar bones unlike mammals.
- Birds lack mesenteric lymph nodes.
- Heart of the birds is comparatively larger.

Blood

- Like reptile, RBCs are nucleated and monocyte is the largest leukocyte in avian blood having a finally granular cytoplasm with a blue grey appearance and the nucleus can be round to bilobate.

- The thrombocyte are equivalent of platelets a unlike the platelets, they are nucleated.

- The heterophile of birds are equivalent of neutrophils of birds.

- The eosinophile are distinct in that they have the intensely red granules in blue staining cytoplasm and the nucleus is also lobed.

- The lymphocytes have a high nucleus-to-cytoplasm ratio; they very in size from small to large.

- The basophils have a non-lobed nucleus with clear cytoplasm containing purple granules.

18. Chelonians

1. The dorsal shell is termed as carapace & ventral shell is termed as plastron.

2. Many shields are present (5 vertebral shields on carapace along with marginal, pleural and supra-marginal shields; In platron, 6 pairs of shields are present, namely gular, humeral, pectoral, abdominal, femoral and anal shields).

3. There is no muscular diaphragm in chelonians but a horizontal connective tissue sheet forms the diaphragmatic membrane, separating lungs dorsally from the coelomic cavity lying ventral to it between the two girdles namely the pectoral girdle and the pelvic girdle.

4. No sternum is present in turtles.

5. Toothless jaws are present in chelonians.

6. Chelonian's eye:

Within the sclera in the anterior hemisphere of the eye ball, a series of bony ossicles are present, thus stiffening the eyes, as in case of birds and this so called sclerotic ring is well developed remarkably in turtles.

7. Tongue is relatively immobile.

8. The stomach is tobacco-pipe like

9. Though urinary bladder is absent in all other reptiles, it is present in chelonions and the kidney is lobed.

10. In case of sea turtles, conical papillae are present in oesophageal region pointing inwards, thus aiding in retention of food in their oral cavity.

11. Caecum & gall bladder are present.

12. Lungs are larger and sac like with many septae, lying against the carapace.

13. Like other reptiles, heart is three-chambered.

14. Chelonions are the egg layers.

19. Crocodiles

1. Skin

a. Dorsum has sales which are ridged or keeled.

b. Ventrum is smooth.

c. Beneath the dorsal epidermal scales, dermal ossifications are present and this contributes to the stiffening of the body covering.

2. Jaw

- The jaw articulation is caudal to the atlanto - occipital joint, hence this reptile is able to open its mouth widely, unlike other creatures.

- Mobility of upper jaw (unlike man) is a special feature in crocodiles.

3. The sclera of crocodile does not contain bony ossicles that are generally encountered in lizards, turtles & birds; however, it may have a cartilage within it (as does the sclera of birds).

4. The nictitating membrane is very thin & transparent and can be drawn over the cornea from the medial to the lateral canthus, like birds.

5. Forelimb has 5 digits and hindlimb has 4 digits.

6. Heart has 3 chambers (2 auricles and 1 ventricle) but at top of incomplete inter-ventricular septum, "Foramen of Panizza" is present.

7. Kidneys are lobulated and the ureters are larger.

8. Penis of crocodile can be palpated by insertion of a finger into the vent.

9. The brain is small compared to the size of the skull and there is no sulci (or) gyri in cerebral hemisphere which is smooth.

10. The tongue cannot be protruded in crocodiles, like the case with elephants.

11. The tongue of estuarine crocodiles has salt glands on it.

12. Pharyngeal pouch is present in posterior aspect of the oral cavity.

13. Stomach has 2 chambers (firstly, the thick walled large chamber and secondly, the thin walled small chamber) and encountering stones in large chamber of crocodile is not a uncommon feature; Though the first chamber of stomach often contains the stones, it is not called "GIZZARD" because, it does not have the circular lining that provides the abrasive actions.

14. Caecum is absent in crocodiles.

15. The structures that open into the cloaca of crocodiles are:

    a. Cranially - the ureters.
    b. Caudally - the genital ducts.
    c. A pair of musk gland opening near vent and these musk glands produce yellowish secretions.

16. Trachea in some species of crocodiles is bent on itself and the long trachea is stiffened by the cartilaginous rings which continue into the bronchi.

17. Lungs are large like any other reptile.

18. Nucleated RBCs and heterophils are the unique features in them.

20. Snakes

- Three chambered heart (2 articles & 1 ventricle) is present.
- Cloaca is the opening for excretion of uric acid and faecal matter.
- Left lung is reduced or absent.
- Right lung is in elongated form.

LOCATION OF ORGANS

Simplistic Classification

From head to vent, divide the length of body into four quarters and anatomical locations of various organs shall be recognised, accordingly.

- Scales are present on skin (like lizard or crocodiles)

Note

Though scaly ant-eater (or) Pangolin are fully covered in scales, there is always the presence of fur or hair materials on them.

- Two jaws are united by ligaments and not by bones.
- External ear-openings are absent.
- Temporal arch (or) inter orbital septum is absent.
- All snakes and lizards lack movable eyelids; but transparent spectacle covers their eyes.
- Liver is very elongate with 2 or 3 lobes.
- Caecum is present in some snakes such as pythons.
- Ecdysis (periodical shedding of skin) is a normal features in snakes or other reptiles.
- Paired hemipenes are present in male snakes.
- Spurs are present in pythons in both male and female.
- Jacabson's organ is a speciality in upper jaw into which the tip of tongue is inserted often to pass the signals of scent.
- Fangs are the modified teeth in snakes belonging to colubridae, elapidae and viperidae.
- Heat-sensitive pits are present on snout region of boas, pythons & pitvipors.
- Urinary bladder is absent.
- Venom glands are located at the outer edge of the upper jaw, immediately below the eye.

21. Amphibians

1. Most of the adult frog and toads have no teeth in lower jaw and have modified tongue to capture the prey.

2. Among amphibians, the caecilians are legless, wormlike and burrowing ones.

3. Tail is resorbed but legs are fully developed & tongue is protrusible and sticky in general.

4. Heart has three chambers (two atria and one ventricle) in adult amphibians.

5. Like any reptile, diaphragm is absent.

6. The unique feature in amphibians is that they store calcium in "para-vertebral lime sacs" which are noticed enveloping the spinal ganglia and these have been used for mineralization of skeleton.

22. Lizards

1. Unlike snakes, ossicles are present within the eyes; Eyelids are usually present except for some geckos and oscellated skink which have snake like spectacles.

2. No. of lizards esp. chameleons have clumps of pigmented cells (chromatophores) in the dermis.

3. Males have femoral pores esp. in iguanas; Femoral and precloacal pores when present are larger in adult male lizards and these are the most reliable means of determining the sex of adult lizards.

4. Skin is thick relatively with ectodermal scales

5. Claws are prominent , large and well developed with sharp points in large lizard species like iguanas and monitors.

6. Teeth are generally peg-like structures; Pleurodont type of dentition is present in lizards (i.e. the teeth are attached to the biting edges of the jaws without sockets)

7. In some iguanid lizards like green iguana , nasal salt glands are present.

8. Tongue is protrusible and prehensile and gets associated with Jacobson's organ.

9. Vocal cords are occasionally present , notably in geckos that can produce loud vocalizations esp. in geckos.

10. Ribs are present usually on all vertebrae except in the tail.

11. Many lizards (and some snakes) which are able to autotomize their tail possess a vertical fracture plane through the body and part of the neural arch of each caudal vertebra and this is a plat of cartilage or connective tissue which develops subsequent to the ossification.

12. Stomach is simple and generally elongated.

13. Caecum is noticed in many species.

14. Thin walled urinary bladder is present in most lizard species.

15. Heart is three chambered (two atria and one ventricle).

16. Like any other reptile , the brain has large cerebrum and cerebellum than that of amphibians and fish.

17. Lizards also lack a diaphragm.

18. Like snakes, hemipenes are present in male lizards.

19. Like any other reptile, the kidneys of the lizards also lack the loop of Henle.

II. POST-MORTEM EXAMINATION

The general practical guide-lines for performing the field necropsies on wild animals, procedure for collection, preservation and despatch of samples for laboratory investigation are detailed below:

Post-mortem examination is an indispensable, intelligent and scientific tool to diagnose disease, by which the morphological changes in tissues and organs are related to specific causes. Disease diagnosis in wild animals is a difficult task, except during an epidemic. Wild animal disease may occur as an outbreak with high morbidity or mortality or as chronic and protracted conditions.

The veterinarian, who conducts the post-mortem should protect himself by wearing mask and other protective clothing to safeguard himself from zoonotic diseases like Rabies, Anthrax, Tuberculosis, Psittacosis etc. If the blood smear from ear vein or smear from throat swelling reveals anthrax bacillus, post-mortem should not be conducted on the carcass, since the organisms are aerobic spore formers (The spores can survive as long as 18 years).

A post-mortem kit should be readily available and contain the following items :

Necropsy documentation	Camera with film
	Field note book
	Balance with spring

	Measuring tape
	Measuring cylinder
Protective clothing	Rubber Apron
	Gum boots
	Gloves
	Coveralls
	Masks
	Eye goggles,
	Face shield
Necropsy equipments	Small and large scissors (Curved)
	Chisel and hammer,
	Small and large knives
	Sharpening stone
	Scalpel and blades
	Bone cutters and saw
	Small and large forceps
	Hand lens
	Spirit lamp or stove
	Bone saw
	Spatula
	Syringe and needles

String

Torch light

Match box / lighter

Specimen containers and sampling materials

Clean glass slides and coverslips

Labelling tape or tags

Water proof labelling pens, pencils

Parafilm or sealing tape

Sprit or alcohol

Cotton and cotton swabs,

Sterilised vial, test tube, Pasteur pipette

Formal saline

Large stainless steel trays

Polypropylene bags with zip lock

Aluminum foil

Ice box with ice

Anticoagulants

Transport materials

Leak proof and break proof boxes

Sealing tapes

Sterile transport media for virus/bacteria

Fixatives

10% buffered formalin

100% acetone for cytology

70% ethyl alcohol for parasites

Disinfecting materials	
	Soap
	Disinfectant solution
	70% ethyl alcohol for disinfecting instruments
	Paper towel
Other equipments	Binocular microscope
	Hand centrifuge

III. GENERAL PROCEDURE TO BE ADAPTED

1. Place or geographical location from where it was recovered.
2. Latest weather conditions - drought, flood, storm etc. and ambient temperature.
3. Common name, zoological name, sex, approximate age and number of animals died.
4. History of the case, duration of illness, morbidity and mortality rate.
5. Weight of the body of dead animal (approximate) - if possible length, girth and other body measurement as required.
6. Mode of transportation of carcass.
7. Date and time of death or found dead.
8. Nutritional status of the dead animal.
9. Signs of struggle or other evidence relating to death.
10. Wounds or other signs of predation.
11. Presence of external parasites - ticks, mites, fleas etc.
12. Fracture, broken or missing teeth, horns, tusk, bruise, bleeding etc.

IV. NECROPSY PROCEDURES IN DIFFERENT SPECIES OF WILD ANIMALS

1. Elephant

General guidelines

- Form a team to perform necropsy in advance.
- Mobilize the team members quickly as and when required.
- Include Veterinary Anatomists, Veterinary Pathologists and Wildlife Veterinarians, from nearby Universities.
- Arrange for standard large animal necropsy instruments.
- Assign specific tasks to team members and conduct post mortem in an organized and systematic way.
- Allocate each team different regions like head, foreleg, hindleg abdomen etc.
- Record all data properly.
- Record all body measurements as required.

Necropsy procedure

- Keep the animal with its back on the ground.
- Position the carcass with rightside down for easy handling of caecum and great colon.

- Remove the head from the body and cut open the cranium to remove the brain.
- Cut the vertebrae and examine spinal cord.
- Examine the eyes and collect one eye for histo-pathology
- Examine the ears.
- Examine the legs and remove them intact
- Examine the nails and sole for lesions.
- Make a mid -ventral incision by cutting through the skin working around the mammary gland in females in pectoral region.
- Expose the sternum
- Examine mammary lymph node
- Continue the midline incision to the perineal region and work around external genitalia in males
- Examine penis and prepuce in males
- Cut the rib at costochondral junction, expose thoracic cavity
- Examine the thoracic cavity contents after separating pleural attachments.
- Remove heart and lungs together with diaphragm.
- Incise pericardium and examine for thickening and increased abnormal contents of pericardial sac.
- Cut open the heart on the right side first and then left side.
- Examine the muscle, endocardium, valves and also major vessels.
- Examine the lung by palpation and after incision for lumps.
- Examine the mediastinal lymph nodes.
- Expose the abdominal cavity and examine the viscera in-situ

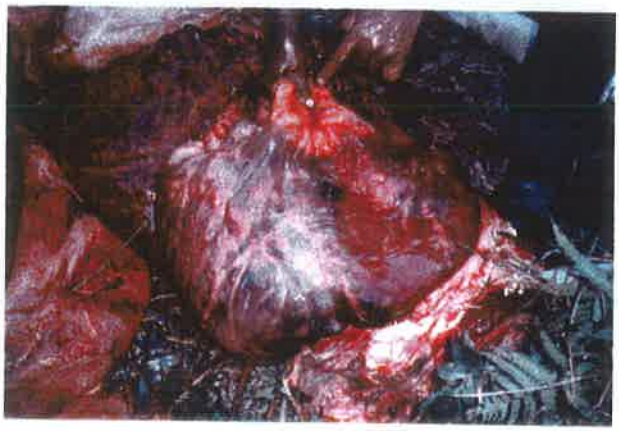
- Examine the cavity for presence of fluid, water, adhesion and displacement of organs.
- Open the pelvic cavity by cutting the pubis.
- Examine the mouth, tongue, tonsil for lesions.
- Examine nasal cavity from trunk for lesions.
- Examine the trunk and nasal passage.
- Examine the intercommunicating canals between nasal passage.
- Remove the tongue, larynx, trachea and oesophagus along with heart and lung by cutting soft tissues and the mandible.
- Examine the thryoid and parathyroid.
- Open the oesophagus , larynx, trachea and examine bronchial lymph node.
- Examine and remove the omentum.
- Examine the spleen and the pancreas and the mesentric lymphnode for lesions. Collect samples from spleen and pancreas.
- Examine the stomach and its contents.
- Examine the liver for lesions.
- Straighten the intestine and cut open and examine the contents.
- Collect sample of contents of gastro-intestinal tract contents for toxicology.
- Collect tissue samples from gastro-intestinal tract for histo-pathology
- Examine the caecum and colon.
- Free the liver from the attachment. Examine the surface and cut surface for lesions

NECROPSY OF ELEPHANT

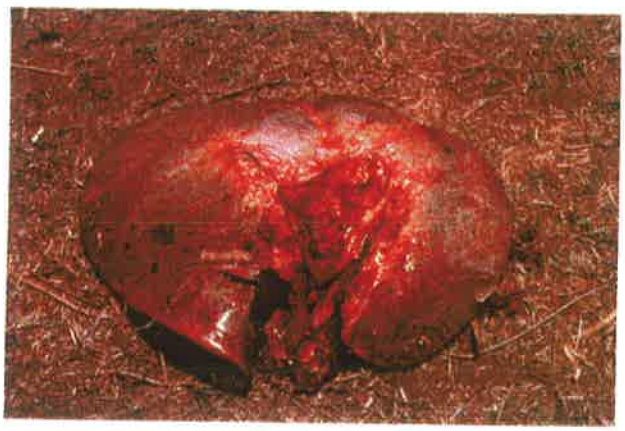
DECAPITATION

OPENING OF THORACIC CAVITY

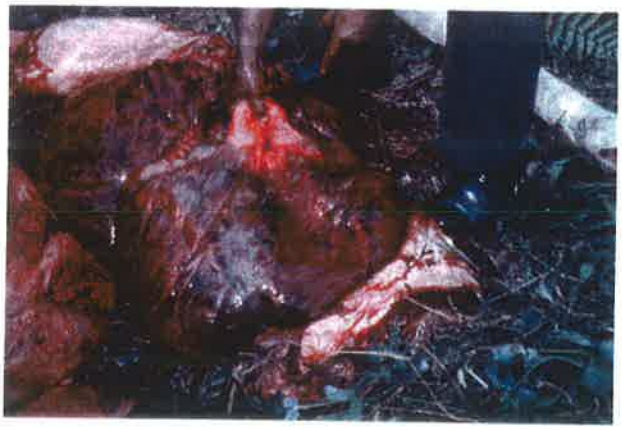
EXAMINATION OF LUNGS



SUB-PLEURAL HAEMORRHAGE

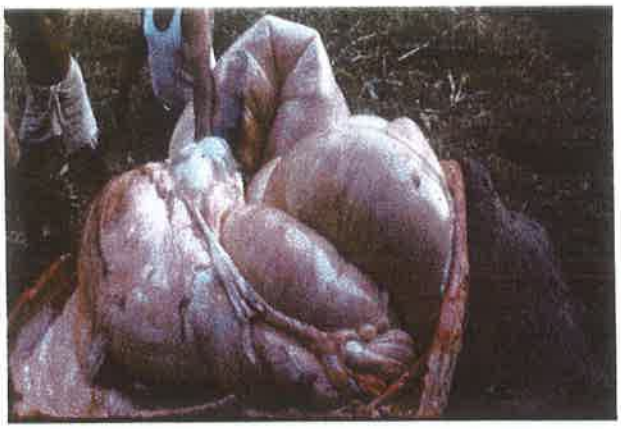


MEDIASTINAL LYMPH NODE EXAMINATION

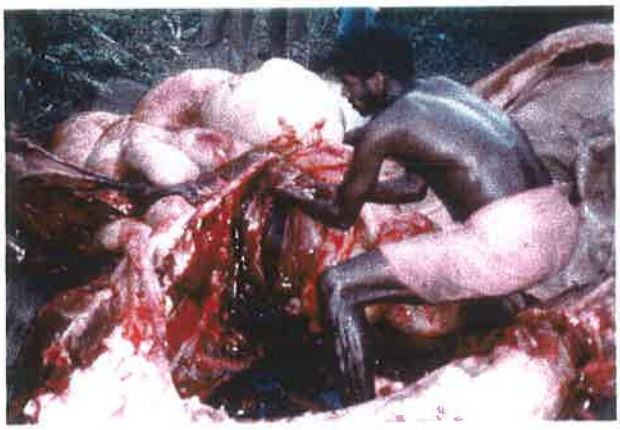


OPENING OF ABDOMINAL CAVITY

EXAMINATION OF ABDOMINAL ORGANS

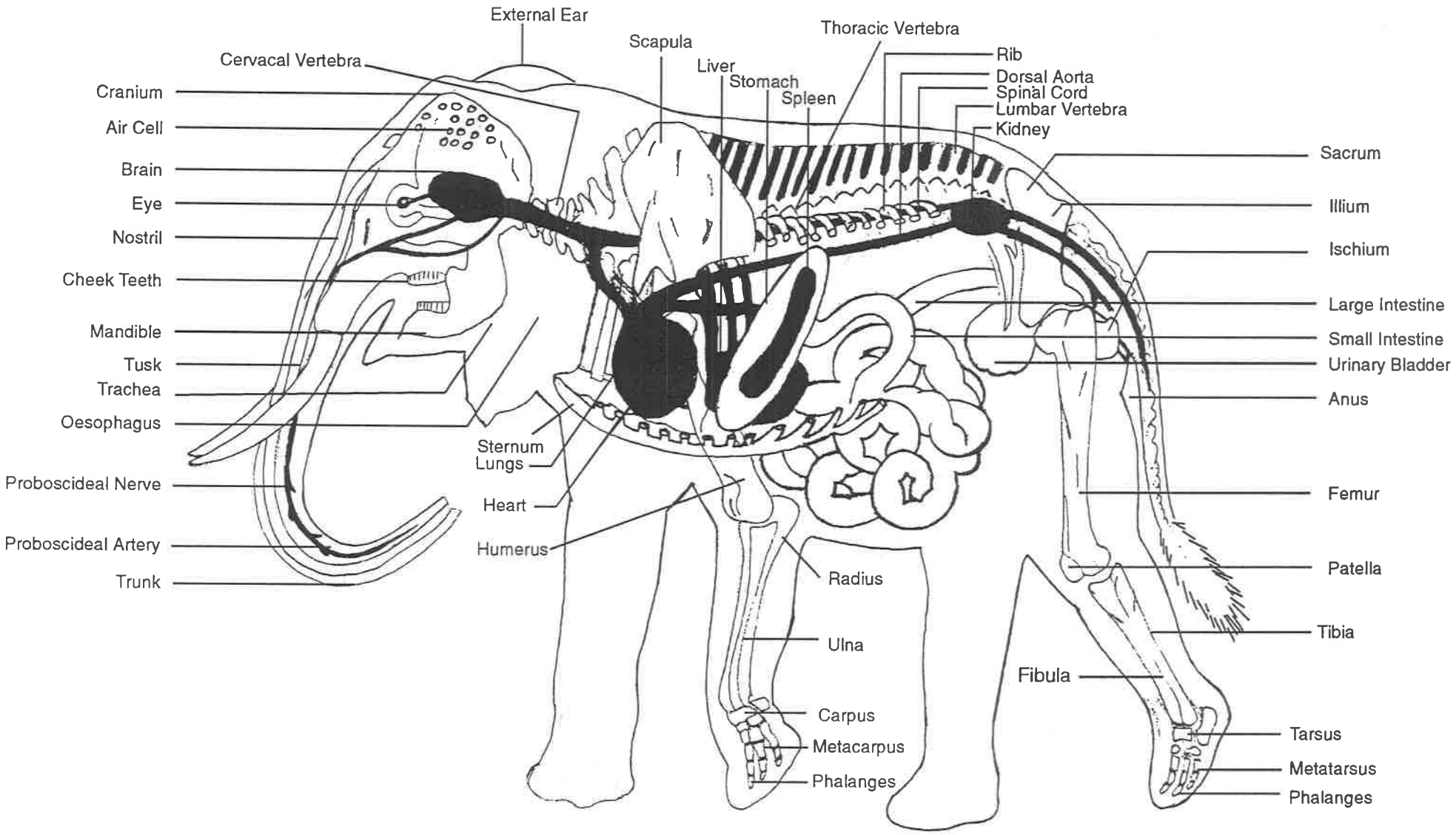


EVISCERATION



GUNSHOT WOUND

ELEPHANT VISCERAL ORGANS



- Measure the length of the tusks and the circumference at the base on either side.
- Examine the adrenal, kidneys and ureter
- Remove the kidneys and then its capsule. After incising the organ longitudinally.
- Examine the cortex, medulla and pelvis.
- Open the urinary bladder and examine the nature of contents.
- Open and examine the uterus, vagina and vulva.
- Locate testes in the sub lumbar area. Incise and examine the cut surface.
- Examine testes and accessory sex glands.
- Examine bone and skeletal muscles particularly shoulder and thigh muscle for lesions
- Dissect sciatic nerve and collect sample.
- Remove a long bone and collect the bone marrow.
- Examine the umbilicus in young ones.
- Examine joints for erosions on articular surface.
- Examine skin for lesion and collect full thickness of abdominal skin.
- Examine temporal glands

2. Carnivores

- Place the animal with the dorsum upon the table
- Note the general condition of the hair/fur
- Examine for external wounds

- Presence of external parasites
- Examine the superficial lymphnodes for enlargement
- Turn the carcass on its left side
- Examine the mammary glands in females
- Make a long incision along the ventral midline from the chin to the tail.
- Examine the prepuce and penis in male
- Examine the umbilicus in neonates
- On the right side separate the skin upto the backbone
- Reflect the right fore limb by cutting through the shoulder muscles
- Reflect the right hind limb by cutting the hip muscles and coxofemoral joints.
- Make an incision from the xiphoid cartilage to the pubis
- Make transverse incisions from the ventral midline to the dorsal extent of the body cavity.
- The abdominal cavity is exposed by removing the flanks.
- Open the right side of the chest cavity by cutting the ribs along the sternum and back bone.
- Examine all organs in situ.
- Incise the pericardial sac and examine its contents.
- Record the quantity, colour and contents of any fluids in all the body cavities.
- Examine oralcavity - the tonsils, pharynx, larynx and salivary glands.
- Examine cervical oesophagus and trachea.

- Remove the stomach and intestine as a unit by cutting the mesentery and cutting across the rectum.
- Examine and collect lymphnodes along the mesenteric attachment.
- Leave the pancreas attached to duodenum and the spleen attached to the stomach.
- Open the stomach and intestine at the end of postmortem examination to prevent contamination of other organs and the necropsy table.
- Note the contents of the stomach and the intestines.
- Collect samples of stomach and intestinal contents for toxicological examination.
- Collect tissue samples from all segments of gastro intestinal tract.
- Collect tissue samples from pancreas.
- Remove and examine the spleen by slicing at multiple sites.
- Remove the liver and open the gall bladder.
- Examine the liver by cutting it across in multiple sites.
- Collect liver samples for histopathology and toxicology.
- Remove and examine adrenals and take samples for histopathology.
- Remove the kidneys and collect samples for toxicology and histopathology
- Remove the reproductive organs and examine (accessory sex glands in male, ovaries, oviduct and uterus in female)
- Examine the larynx, trachea, thyroid and oesophagus and remove with the lungs.

- Examine the thymus if present and collect samples.
- Open the trachea, bronchi and other airways.
- Open the Oesophagus.
- Examine mediastinal lymphnodes
- Examine the lungs for lesions and collect samples for histopathology
- Open the chambers of the heart and examine the heart valves.
- Open the major blood vessels
- Examine the eyes and nostrils for lesions and abnormal discharges
- Remove an eyeball for histology.
- Remove the tongue from its base by cutting between the lower jawbones.
- Examine mouth, tonsils, teeth and lymphnodes.
- Separate the skull from the neck at the junction of the vertebra
- Remove the skin from the top of the head and the skull.
- Strip off the muscles to expose the skull.
- Break open the skull with the help of a chisel and hammer.
- Cut the meninges and expose the brain.
- Dissect the brain into two halves.
- Preserve one half in buffered formalin and split the other half into containers for virology and toxicology.
- If Rabies is suspected, dissect the hippocampus major.
- Collect pieces of the hippocampus major in glycerine for biological/virololgical examination and formal saline for histopathology.

NECROPSY OF TIGER

CARCASS OF A CAPTIVE TIGER

EXAMINATION OF ORAL MUCOSA

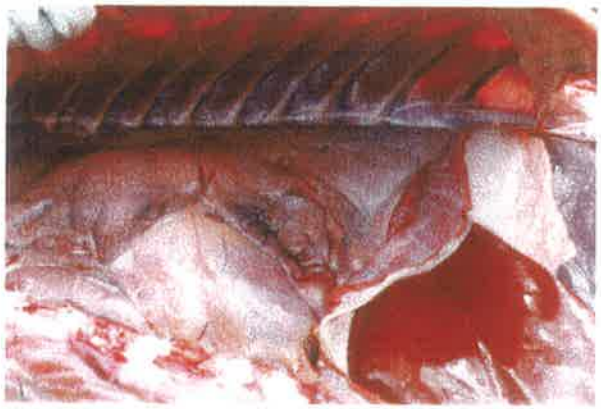
EXAMINATION OF CONJUNCTIVA

PREPARATION OF BLOOD SMEAR

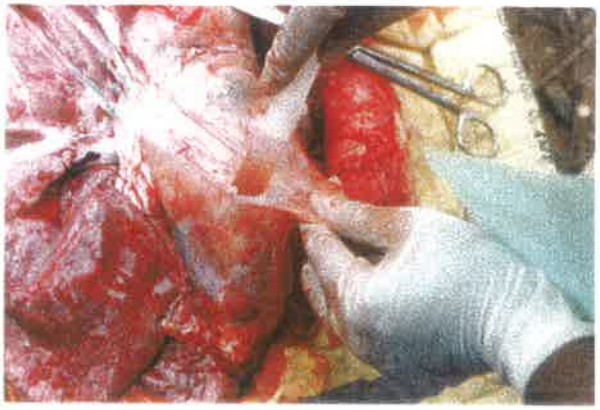
EXAMINATION OF MUSCLES IN SHOULDER

EXAMINATION OF VENTRAL REGION

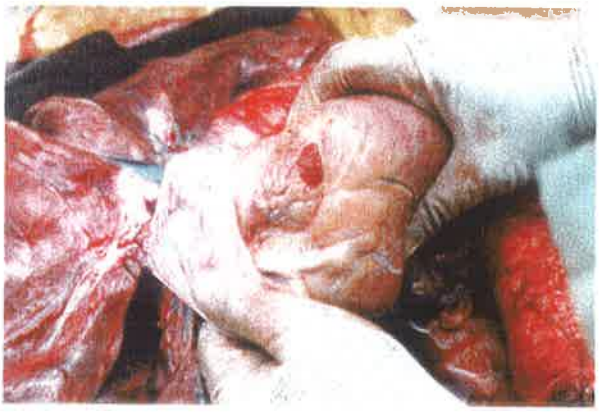
EXAMINATION OF VISCERAL ORGANS IN-SITU



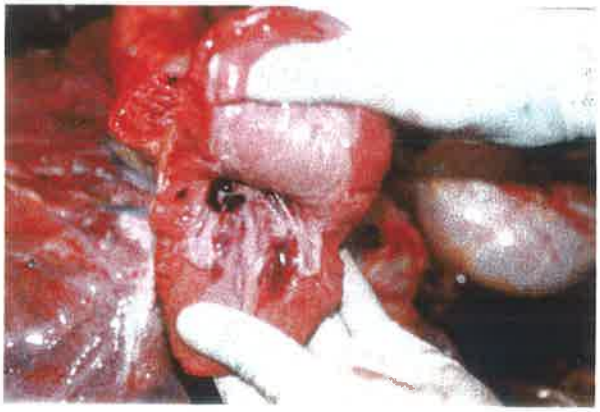
PERICARDIAL FLUID COLLECTION



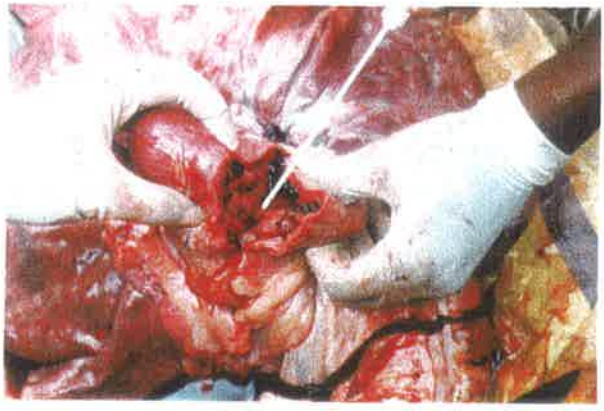
EXAMINATION OF HEART



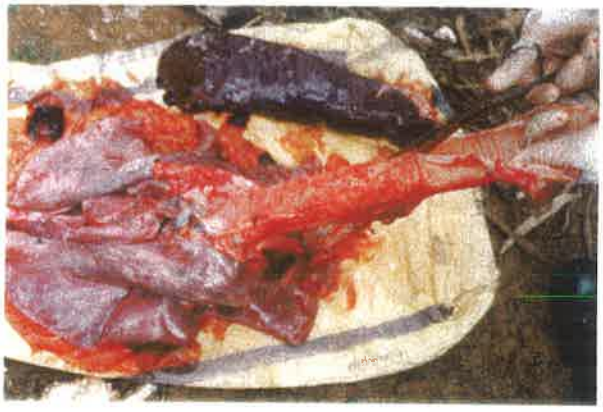
EXAMINATION OF ENDOCARDIAL SURFACE



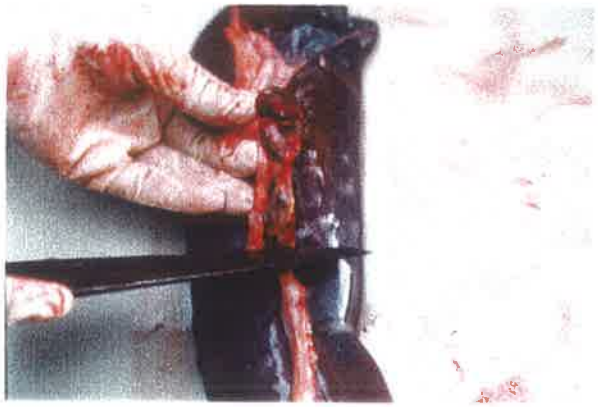
COLLECTION OF HEART BLOOD SWAB



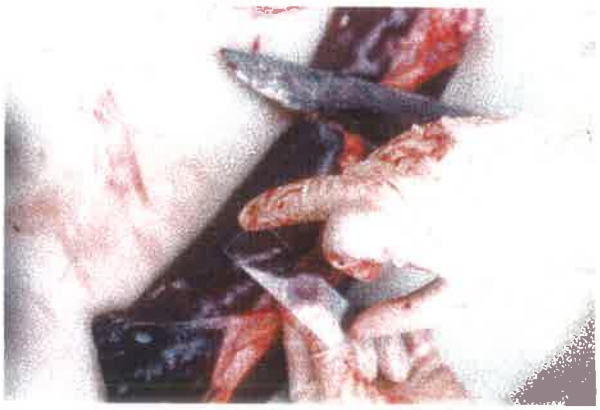
EXAMINATION OF TRACHEA



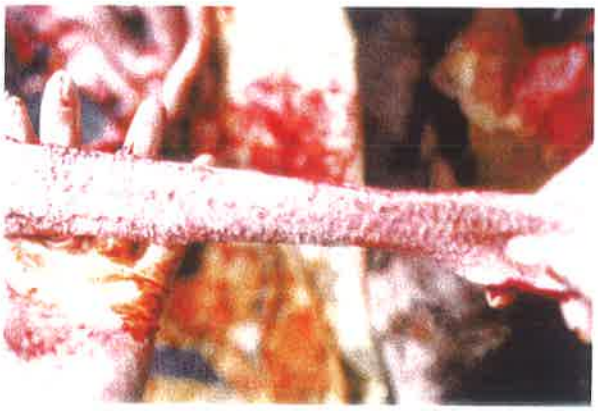
SPLENIC LYMPHNODE EXAMINATION



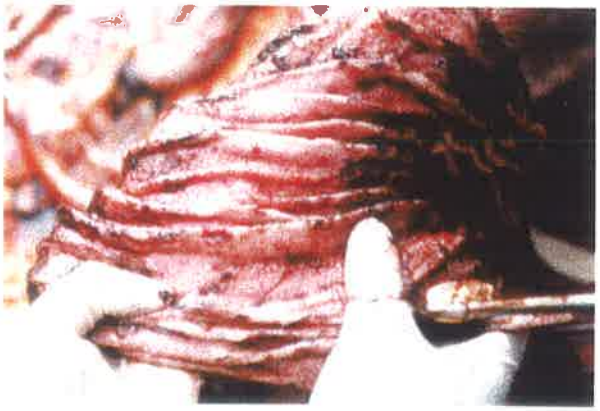
IMPRESSION SMEAR FROM SPLENIC LYMPHNODE



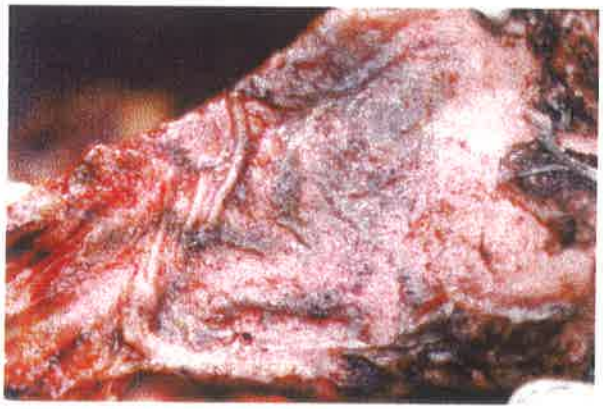
EXAMINATION OF OESOPHAGUS



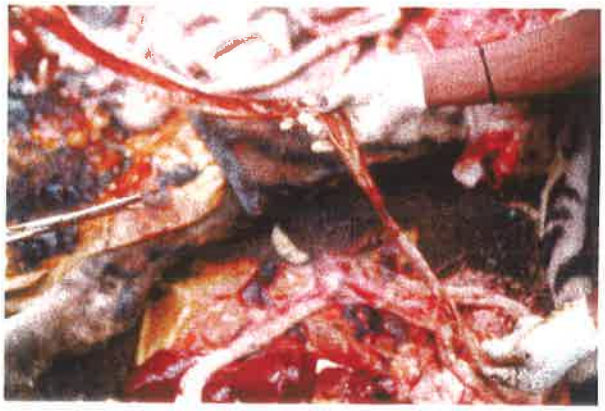
EXAMINATION OF GASTRIC MUCOSA



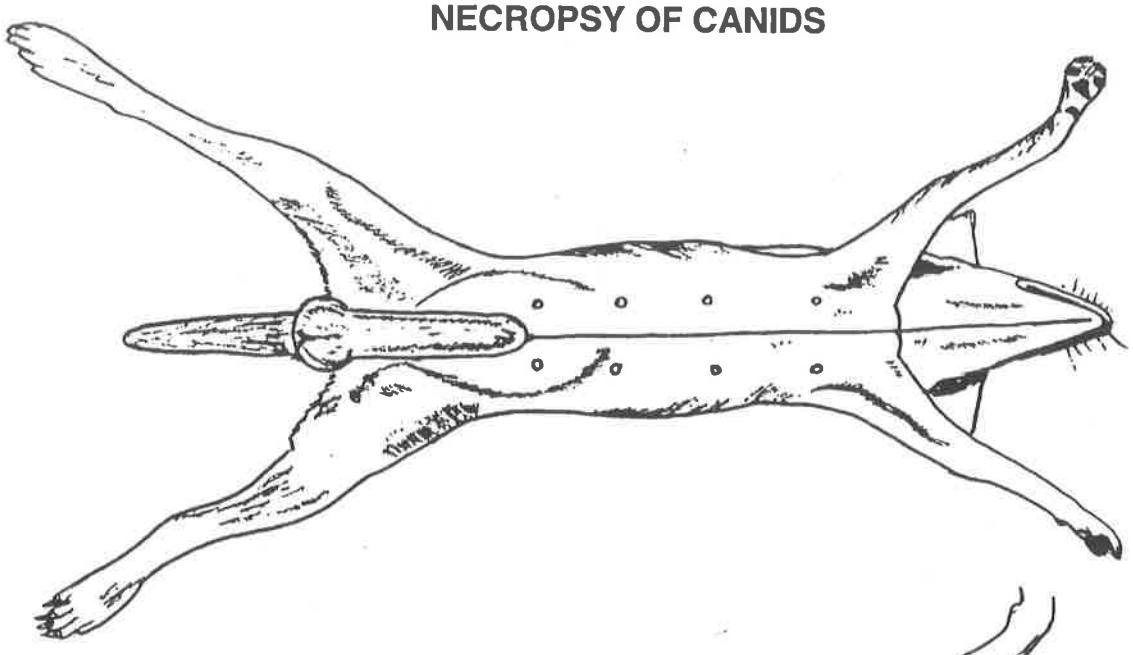
EXAMINATION OF PYLORIC REGION

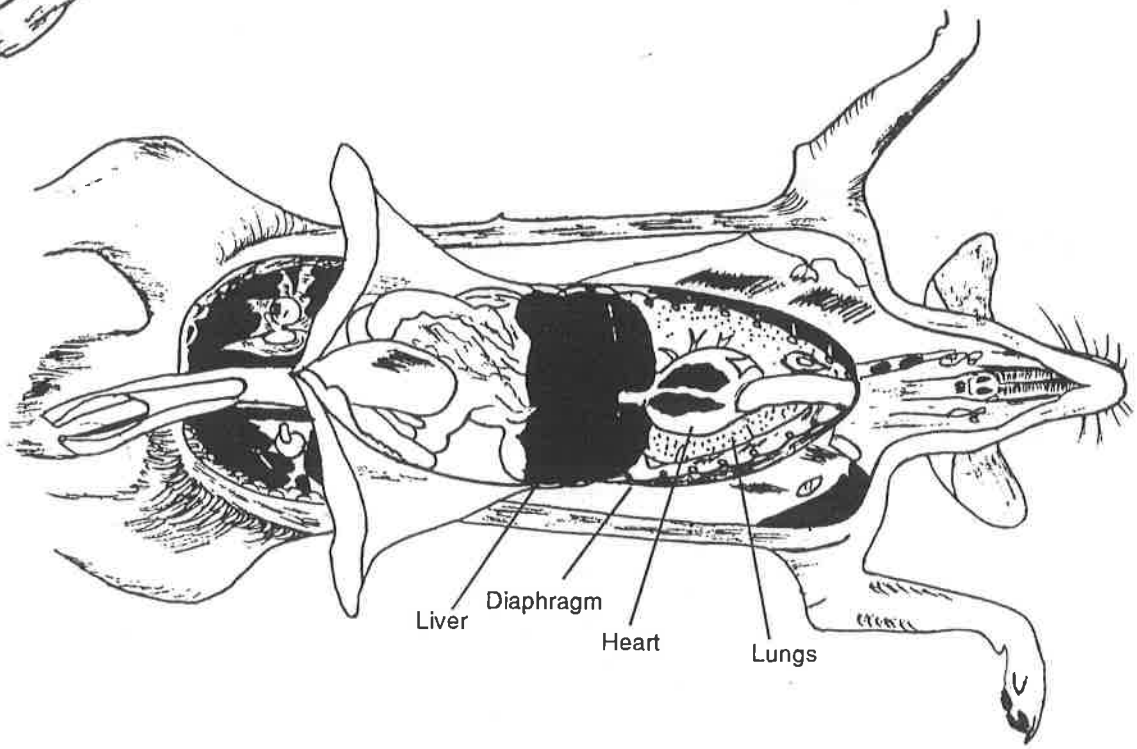


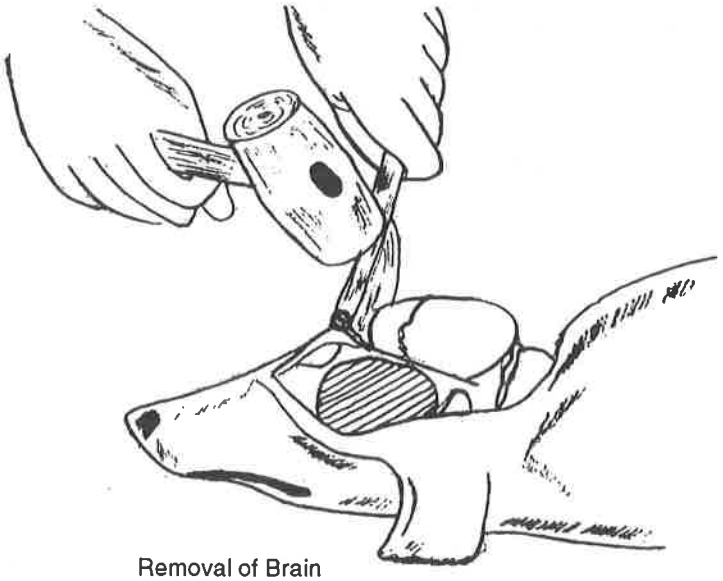
EXAMINATION OF INTESTINAL LUMEN



NECROPSY OF CANIDS

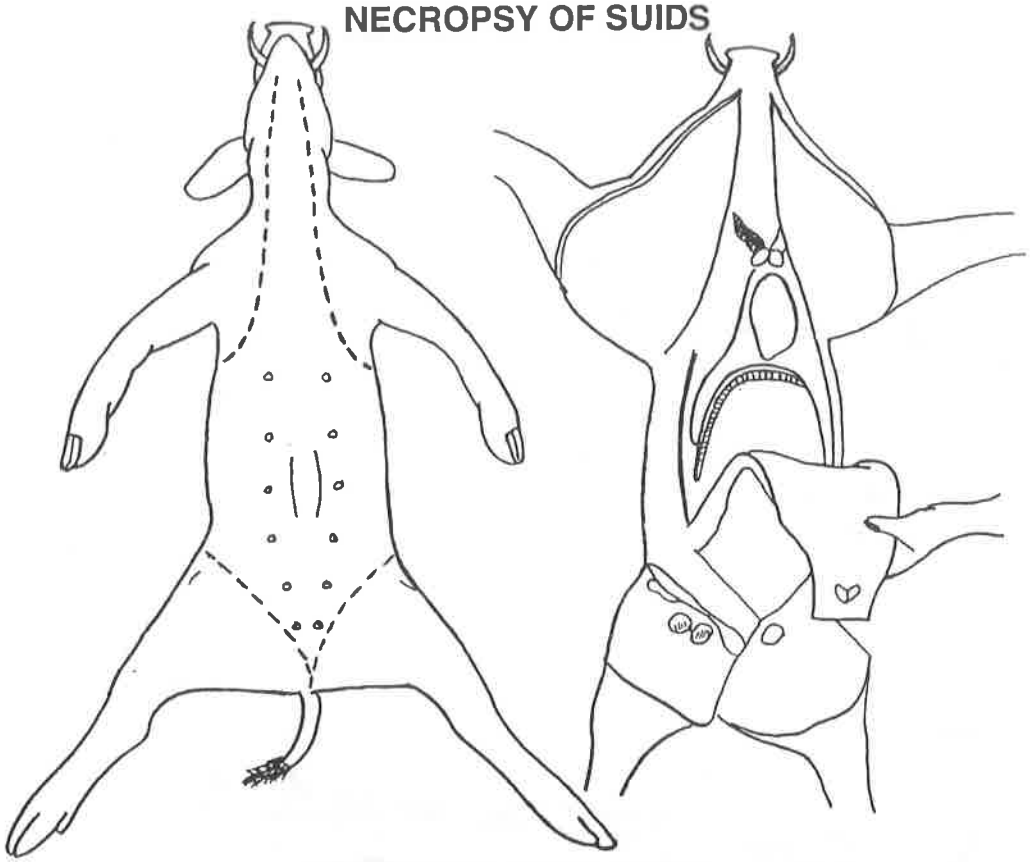






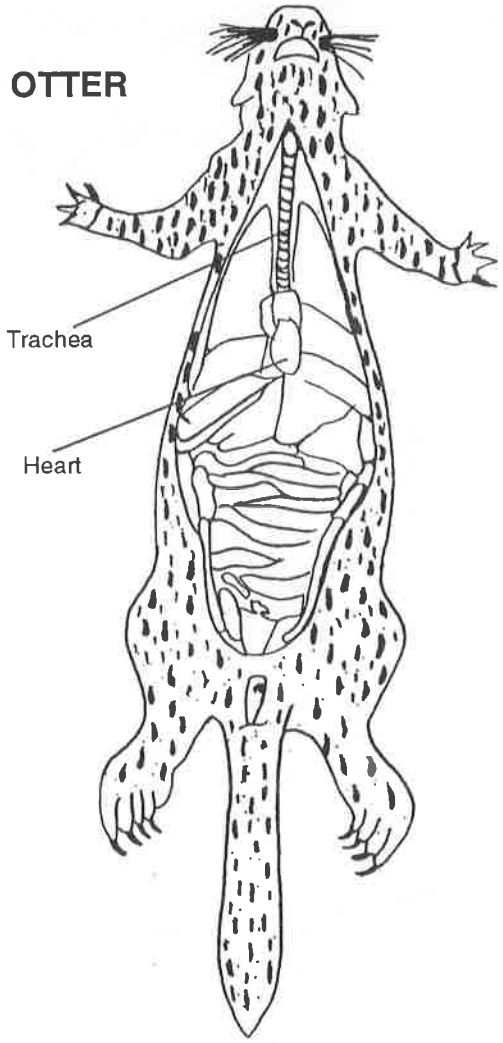
Removal of Brain

NECROPSY OF SUIDS



Dotted Line Indicates Line of Incision

NECROPSY OF OTTER



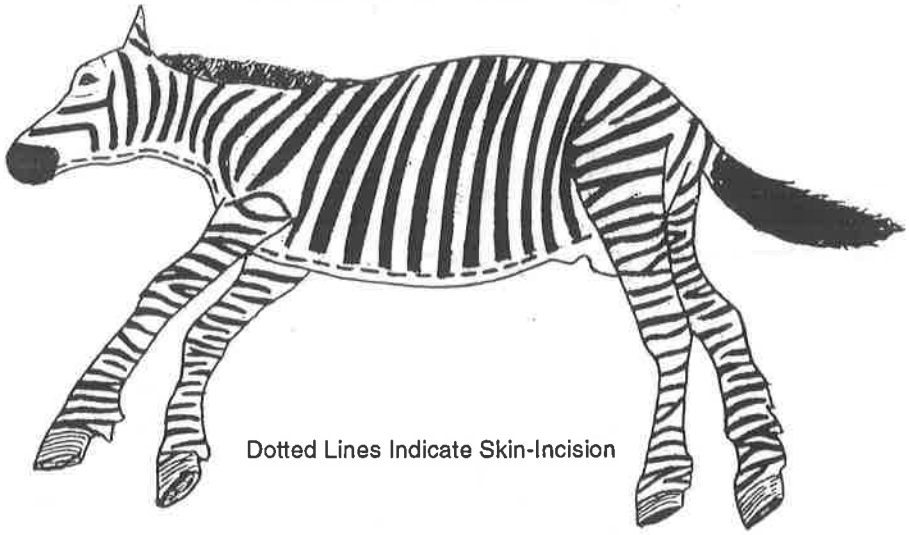
- If it is not possible to dissect the hippocampus major, send one half of brain for virology.
- Cut the vertebrae and examine the spinal cord.
- Examine the diaphragm and skeletal muscles.
- Examine the long bones, costal bones etc. for lesions.
- Remove a long bone from the leg and collect the bone marrow for histo-pathology and microbiology.

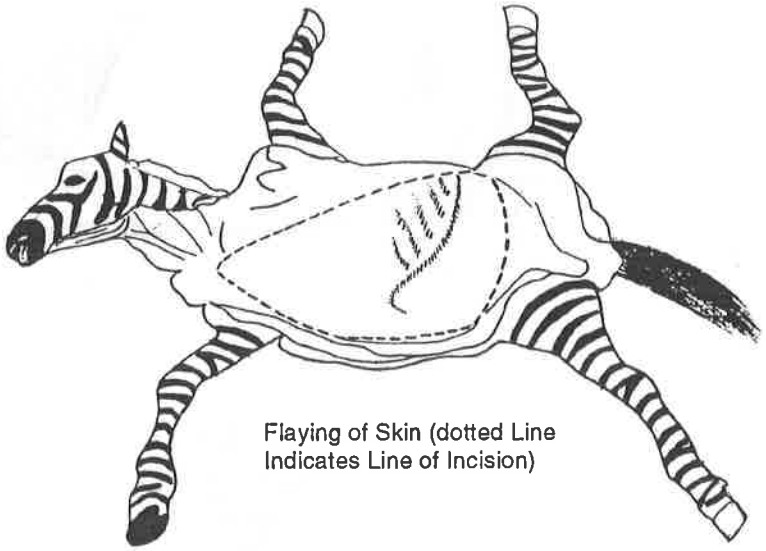
3. Ungulates (Procedure of external examination is common to all species)

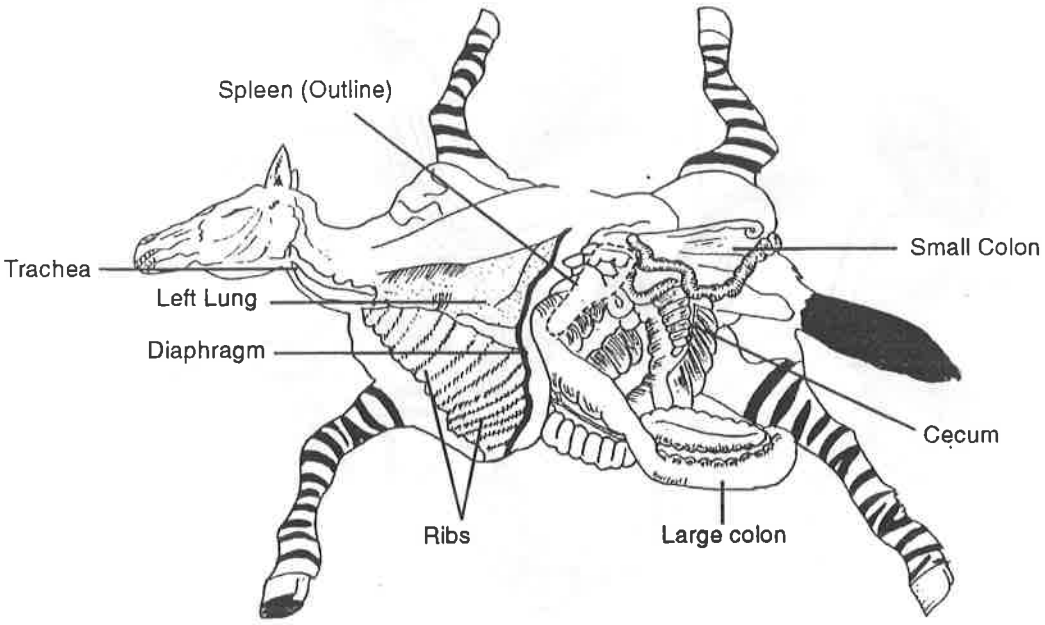
- Keep the animal with its back on the ground.
- In ruminants, place the animal on the left side so that the rumen is away from the prosecutor.
- Position the equine carcasses with right side down for easy handling of caecum and great colon.
- Make incision on the midline from between jaws to the perineal region, working around the mammary gland in females and external genitalia in males.
- Remove the mammary gland with skin intact, examine for symmetry, swelling, lumps and firmness.
- Examine the mammary lymph nodes.
- Examine the penis and the prepuce in males.
- Examine the umbilicus in young ones.
- Lift the right legs away from the body.
- Extend the incision to the medial side of each leg.

- Separate front and hind right legs by cutting muscles and hip joints.
- Extend the incision from the sternum to the pelvis and open the abdominal cavity.
- Expose the thoracic cavity by cutting ribs and at costochondral junction and vertebrae.
- Examine body cavities for fluid contents, adhesions and displacements of visceral organs.
- Open the pelvic cavity by cutting the pubis.
- Incise the pericardium and examine for thickening and abnormal contents of pericardial sac.
- Examine the mouth, tongue, tonsil salivary gland, pharynx, larynx and thyroid for lesions.
- Examine the nasal cavity for lesions.
- Remove the omentum
- Examine the spleen, pancreas and the mesenteric lymph nodes for lesions.
- Open and examine the rumen, reticulum, omasum and abomasum for lesions and abnormal contents.
- Examine the liver for lesions.
- Open the gall bladder and bile duct for lesions, worms, gallstones etc.
- Straighten the intestine cut open and examine.
- Collect sample of contents of Gastro -intestinal tract for toxicology.

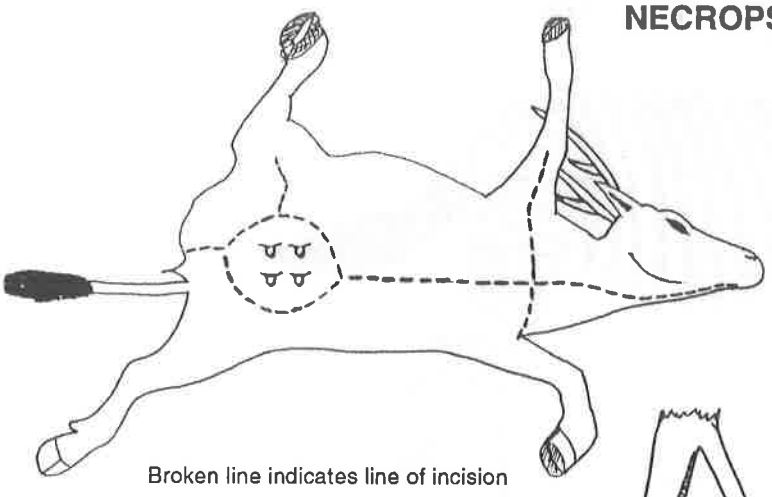
NECROPSY OF EQUID



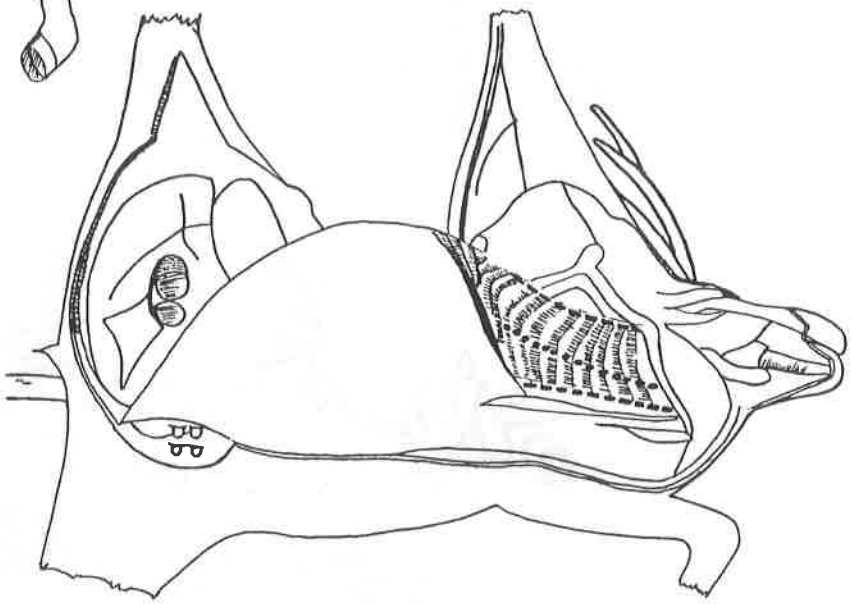


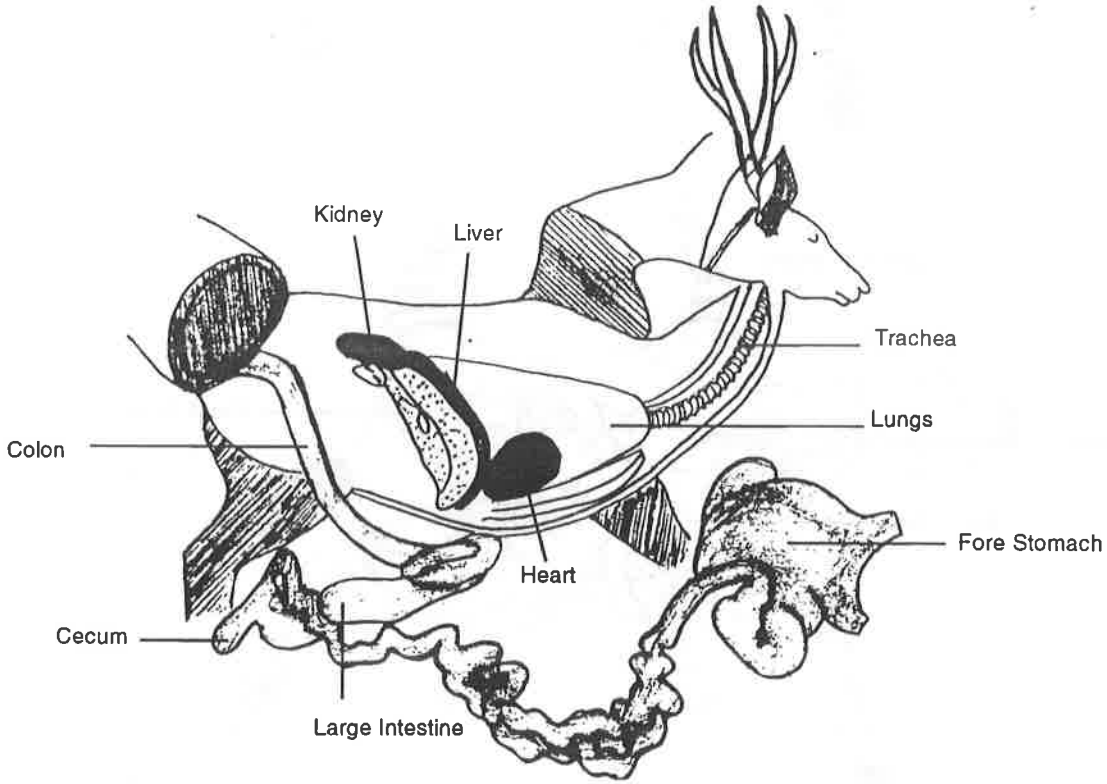


NECROPSY OF CERVID

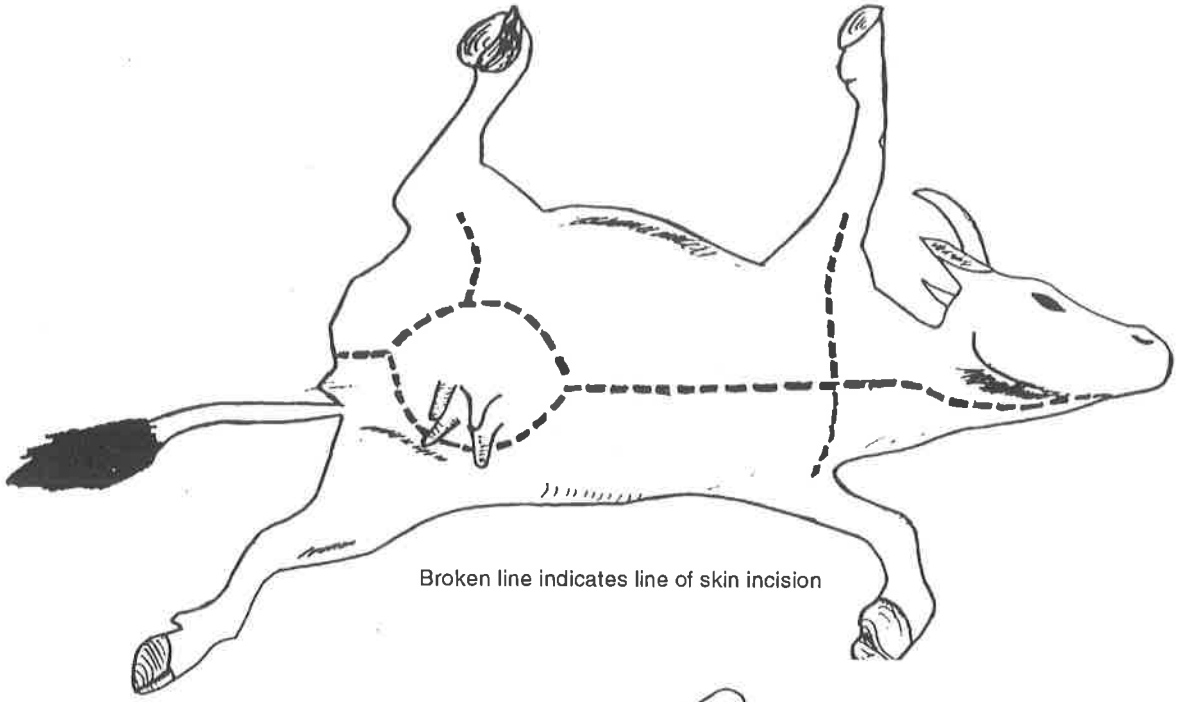


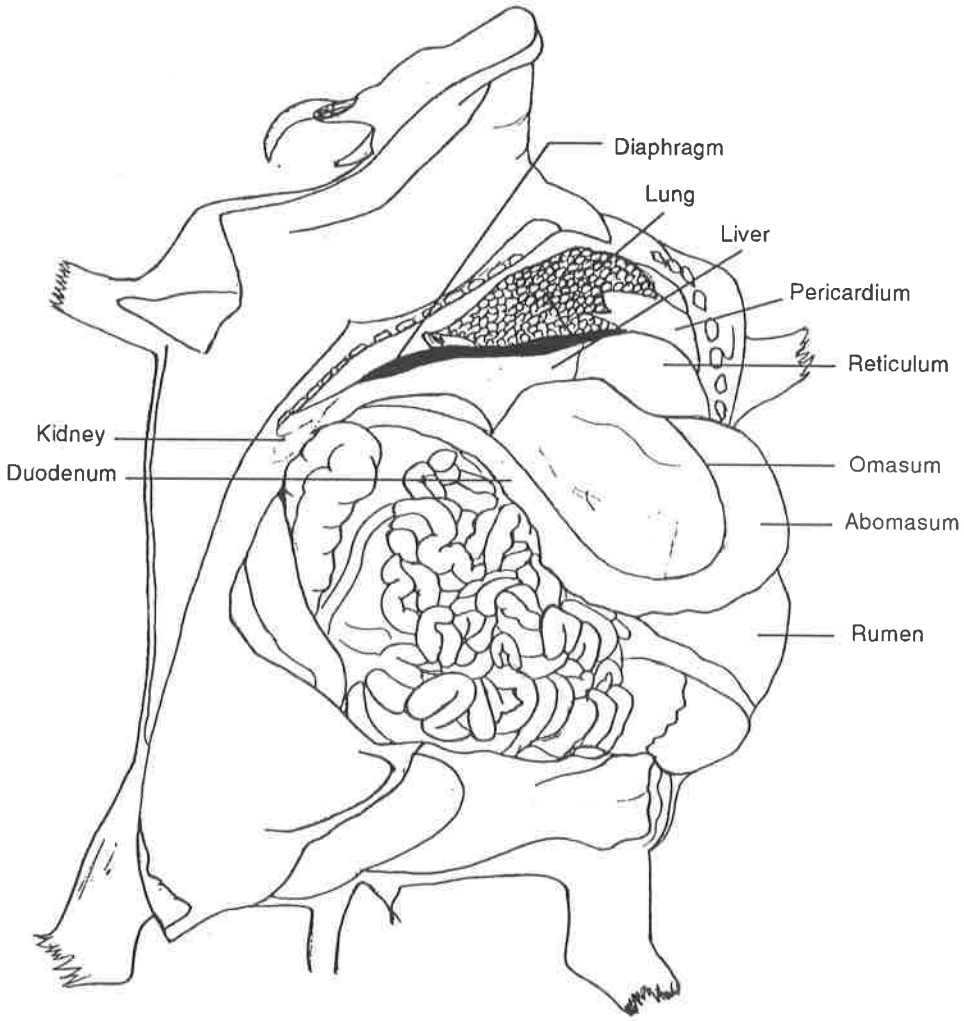
Broken line indicates line of incision





NECROPSY OF BOVID





- Collect tissue samples from Gastro -intestinal tract for histo-pathology.
- Examine and collect samples from pancreas.
- Examine the spleen for lesions, slice and collect samples
- Free the liver from the attachment examine the surface and cut surface for lesions.
- Examine the adrenals, kidneys, ureters etc.
- Remove the kidney and then remove the capsule after incising the organ longitudinally.
- Examine the cortex, medulla and pelvis.
- Open the urinary bladder and examine the nature of contents.
- Open and examine the uterus, vagina and vulva.
- Examine the testis and accessory sex glands.
- Examine the thyroid and parathyroid.
- Remove the tongue, larynx, trachea and oesophagus along with heart and lung by cutting soft tissues and the mandible.
- Examine mouth and tongue.
- Open the oesophagus, larynx and trachea and examine.
- Examine the lung by palpation and after incision for lumps.
- Examine the heart
- Cut open the heart on right side first and then left side.
- Examine the myocardium, endocardium, valves and also major vessels.
- Examine the eyes and collect one eye for histo-pathology
- Separate the head from the neck.
- Remove the skin and muscles to expose the skull.

- Break open the skull to expose brain.
- Cut the meninges, dissect out the brain and collect samples.
- Cut the vertebrae and examine the spinal cord.
- Examine the bones and skeletal muscles particularly shoulder and thigh muscles for lesions.
- Remove a long bone and collect bone marrow

4. Non-human Primates

- Read the case history and ascertain the identity of carcase
- Examine the hair coat.
- Examine the skin for any wounds / lesions
- Flay the skin completely
- Examine the subcutis for the presence of fat, dehydration , bruises etc.
- Examine the scent glands
- Examine the umbilicus for the patency and vascular changes in neonates
- Examine the mucous membrane for its colour and exudates
- Examine the natural orifices for any discharge
- Examine oral cavity including tongue, cheek pouches, pharynx and tonsils
- Examine the skeletal musculature for its colour and slice the muscles from its attachments with bones
- Examine the bones for any malformation or fracture; Disarticulate the femur from acetabular cavity.

- Split the femur longitudinally and examine the bone marrow
- Open the joint cavities and examine the synovial fluid, synovial membrane and articular surfaces
- Remove the front legs at the attachment of shoulder blade with thorax
- Disarticulate the head at the atlanto-occipital joint
- Examine the larynx, laryngeal air sacs for its colour and presence of exudates
- Examine the mandibular and parotid salivary glands, thyroids, parathyroids and cervical lymph nodes.
- Open the cervical oesophagus and examine the luminal content and mucosal changes.
- Make a nick at the ventral abdominal wall and extend forward upto the thoracic inlet through costo-chondral junction
- Examine the abdominal cavity for the presence of any extensive abnormal peritoneal fluid , position of organs and adhesions.
- Examine the thoracic cavity for effusions, adhesions or haemorrhages
- Examine the mediastinal fat
- Locate the thymus and examine it for its size and colour
- Examine the heart for its size, shape, epicardial surface colour and coronary fat.
- Incise the heart through auriculo-ventricular aperture and examine the endocardium for vascular changes and thrombus.
- Open the aorta and pulmonary artery and examine the intimal colour and surface.

- Open the thoracic oesophagus fully and look for its contents and mucosal lesions
- Cut open the tracheobronchial tree and examine the luminal contents and mucosal lesions
- Note the colour of the lungs; Palpate the parenchyma for the presence of any nodule or lump.Incise the parenchyma transversely at 3-4 places to examine the cut surface.
- Remove the bronchial an mediastinal lymph nodes and note the size, colour and cut surface lesions.
- Examine the omental, mesenteric and perirenal fat for its quantity, colour and presence of lumps.
- Remove the abdominal organs enmasse.
- Make a nick over the duodenum and open it fully
- Press the gall bladder and look for its drainage at the duodenal mucosa.
- Remove the liver with gall bladder.
- Examine the liver for its colour, size, borders and make incision on the parenchyma in different lobes and examine the cut surface.
- Open the gall bladder and examine the bile.
- Remove the pancreas and examine the surface for any white spots, haemorrhages etc.
- Open the stomach and look for its contents and examine the mucosa.
- Examine the mesenteric lymph nodes for its colour, size and cut surface lesions.

- Open the intestine to its full length and examine the contents and look for any parasites; Examine the mucosa for its colour and thickness
- Remove the kidneys and slice them longitudinally
- Peel the capsule and examine the cortical surface
- Note the thickness and colour of cortex and medulla
- Examine the pelvis for its luminal size, contents and mucosal lesions
- Open the bladder and examine the urine
- Open the urethra and examine the mucosa
- Open the female reproductive tract in females from vagina to tip of uterine horns
- Examine the endometrium for its colour, thickness etc.
- Examine the male accessory sex organs (prostate and seminal vesicles)
- Open the scrotal bag and examine the cavity of tunica vaginalis for the presence of fluid, blood or prolapsed portion of intestine
- Incise the testis longitudinally and transversely for its colour and note the consistency
- Open the abdominal aortas, caudal vena cava and iliac vessels and look for any thrombus
- Remove the skull on the dorsum of brain
- Examine the meninges for its colour, thickness etc.
- Remove the brain intact by scooping it from the cranial cavity.
- Slice the brain in 3-4 places transversely and examine the cut surface for any lesion-congestion, haemorrhages, cyst or abscess

- Examine the pituitary at the base of brain
- Remove the spinal cord if nervous signs are reported and take pieces from suspected portions for histo-pathological examination
- Examine the peripheral nerves-brachial plexus and sciatic nerves for their thickness and colour

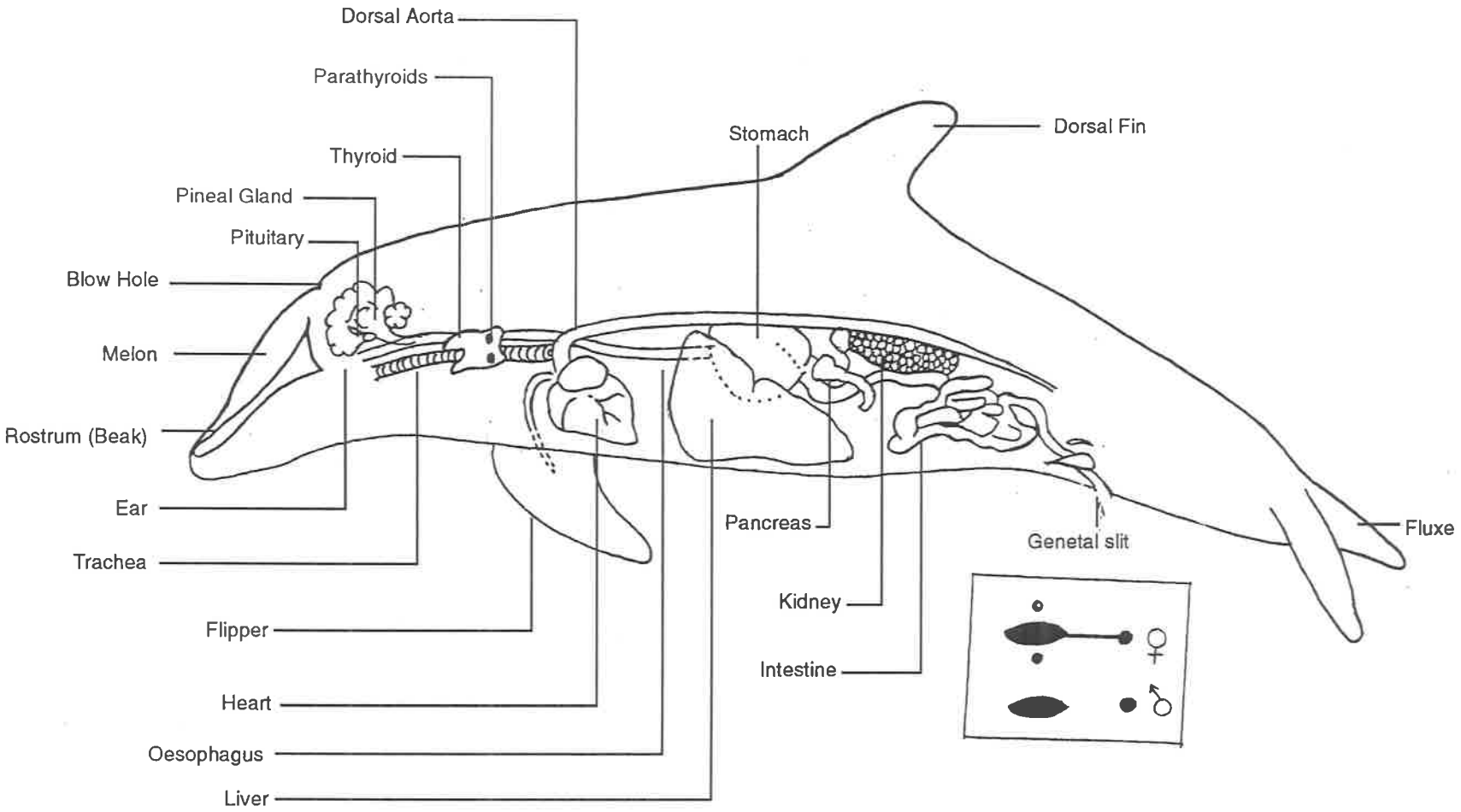
5. Marine mammals (Dolphins)

- Collect the case history relating to the clinical illness and laboratory examinations carried out till death.
- Ascertain the identity of carcass
- Examine the mucous membrane for its colour and exudates
- Examine the natural orifices for any discharge
- Examine the skin (flippers, tail and dorsal fin) for any wound
- Incise the ventral skin from mouth to genital opening
- Flay the skin completely
- Examine the musculature for its colour , stiffness etc.
- Slice the muscles from its attachments with bones
- Examine the bones for any malformation or fractures
- Examine the nasal passage, larynx and tracheobronchial tree for its colour and presence of exudates.
- Examine the mandibular, parotid salivary glands, thyroids, parathyroids, cervical lymph nodes
- Open the cervical oesophagus and look for luminal contents and mucosal changes

- Examine the abdominal cavity for the presence of any excessive abnormal peritoneal fluid , position of organs and adhesions.
- Examine the thoracic cavity for effusions, adhesions or haemorrhage.
- Examine the mediastinal fat
- Locate the thymus and examine for its size.
- Examine the heart for coronary fat, size and shape and epicardial surface for colour.
- Incise the heart longitudinally and examine the endocardium for vascular changes and thrombosis.
- Open the aorta and pulmonary artery and examine the intima colour and surface.
- Open the thoracic oesophagus fully and look for its contents and mucosal lesions.
- Note the colour of the lungs; Palpate the parenchyma for the presence of any nodules or lungs; Incise the parenchyma transversely in 3-4 places.
- Remove the bronchial and mediastinal lymph nodes and note the size , colour and cut surface for lesions.
- Examine the omental, mesenteric and perirenal fat for its quantity, colour and presence of lumps.
- Remove the abdominal organs enmasse
- Examine the liver for its colour, size, borders and make incision on the parenchyma in different lobes and examine the cut surface.
- Remove the pancreas & examine the surface for any white spots, haemorrhages etc.

- Open the forestomach , fundic compartment, pyloric compartment and ampulla of stomach and examine its contents and the mucosa.
- Examine the mesenteric lymph nodes for its colour , size and cut surface lesions.
- Open the intestine to its full length and examine the contents and look for any parasites; Examine the mucosa for its colour.
- Remove the kidneys and slice them longitudinally; Peel the capsule and examine cortical surface and note the thickness and colour of cortex and medulla; Examine the pelvis for its luminal size, contents and mucosa; Open the bladder and examine the urine. Open the urethra and examine the mucosa.
- Open both the uterine horns from genital opening and examine the contents and mucosa.
- Incise the testis (located lateral to the kidneys) longitudinally and transversely and examine for the colour and note the consistency.
- Open the abdominal aorta, caudal venal cava and iliac vessels and look for any thrombus
- Remove the skull on the dorsum of brain. Examine the meninges for its colour , thickness etc. Remove the brain intact by scooping it from the cranial cavity; Slice the brain in 3-4 places, transversely and examine the cut surface for any lesion- congestion, haemorrhages, cyst or abscess.
- Examine the pituitary at the base of brain

VISCERA OF DOLPHIN



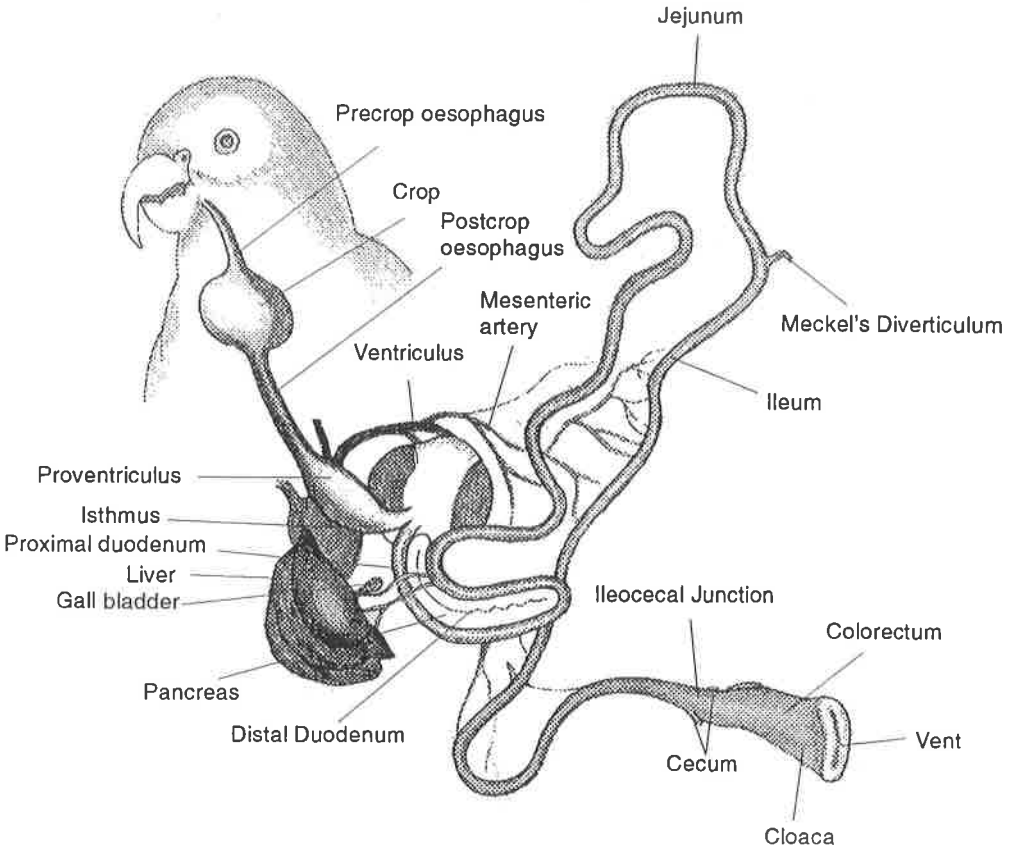
- Remove the spinal cord if nervous signs are reported and take pieces from the suspected positions for histo-pathological examination
- Examine the peripheral nerves - brachial plexus and sciatic nerves for any abnormality(change in thickness and colour)

6. Aves

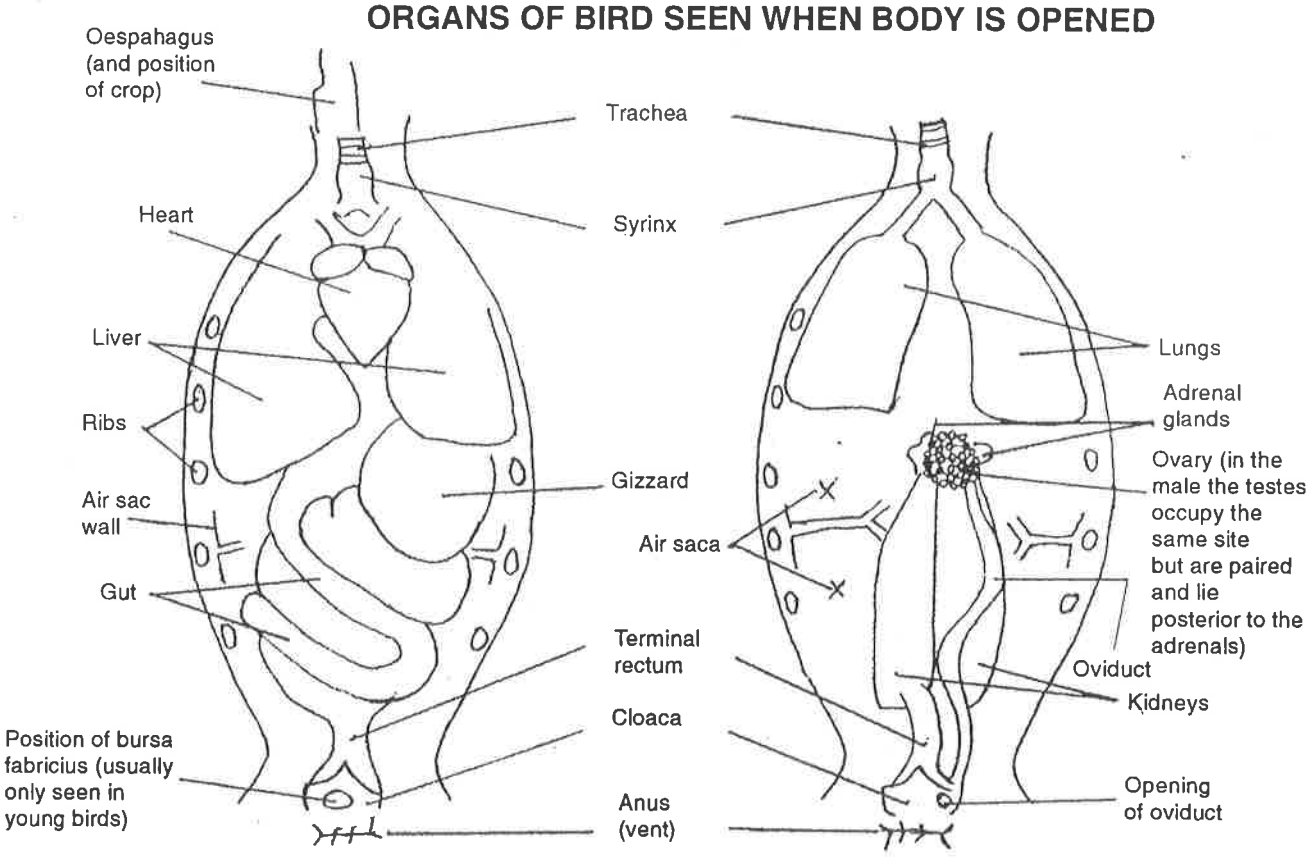
1. Examine the body condition.
2. Examine the beaks, eyes, plumage, skin, leg scales etc for injury.
3. Record the body weight.
4. Examine for external parasites and examine for any fracture , joint swelling, loosening, discharge from natural orifices.
5. Keep the birds on its back with wings and legs extended.
    - Wet the skin with a disinfectant solution
    - Make incisions between the abdomen and leg on either side.
    - Break open the hip joint by dislocating the head of each femur from acetabulum.
    - Cut the skin between the keel and the vent transversely.
    - Peel off the skin over the breast muscles, abdomen and legs.
    - Free the crop from clavicular space by blunt dissection.
    - Incise the abdominal wall transversely just below the keel bone.

- Insert the scissors blunt edge into the abdominal cavity and cut through the sides of the rib cage extending up to corocoid and clavicular bone on either side.
- Lift the Sternum.
- Remove the sternum to expose the internal organs.
- Examine the muscles of thigh and sternum.
- Extend the incision along the mid abdominal wall up to cloaca.
- Remove the liver with gall bladder
- Remove the spleen.
- Make transverse incision on the oesophagus just anterior to the proventriculus.
- Gently pull the stomach and intestine in one piece
- Cut through the dorsal and ventral attachment of oviduct and open it from the cloaca to the infundibulum.
- Examine the airsacs by opening them
- Incise the pericardial sac and observe the pericardial fluid.
- Remove the heart by gentle pulling and cutting the major blood vessels arising from the heart.
- Remove the lungs from the thoracic wall by blunt dissection and cutting through the dorsal attachment and anterior to the syrinx.
- Make transverse incision in the upper beak to expose the nasal cavity.
- Expose the paranasal sinus by incising the openings on either side.

VISCERA OF PSITTACINE BIRD



ORGANS OF BIRD SEEN WHEN BODY IS OPENED

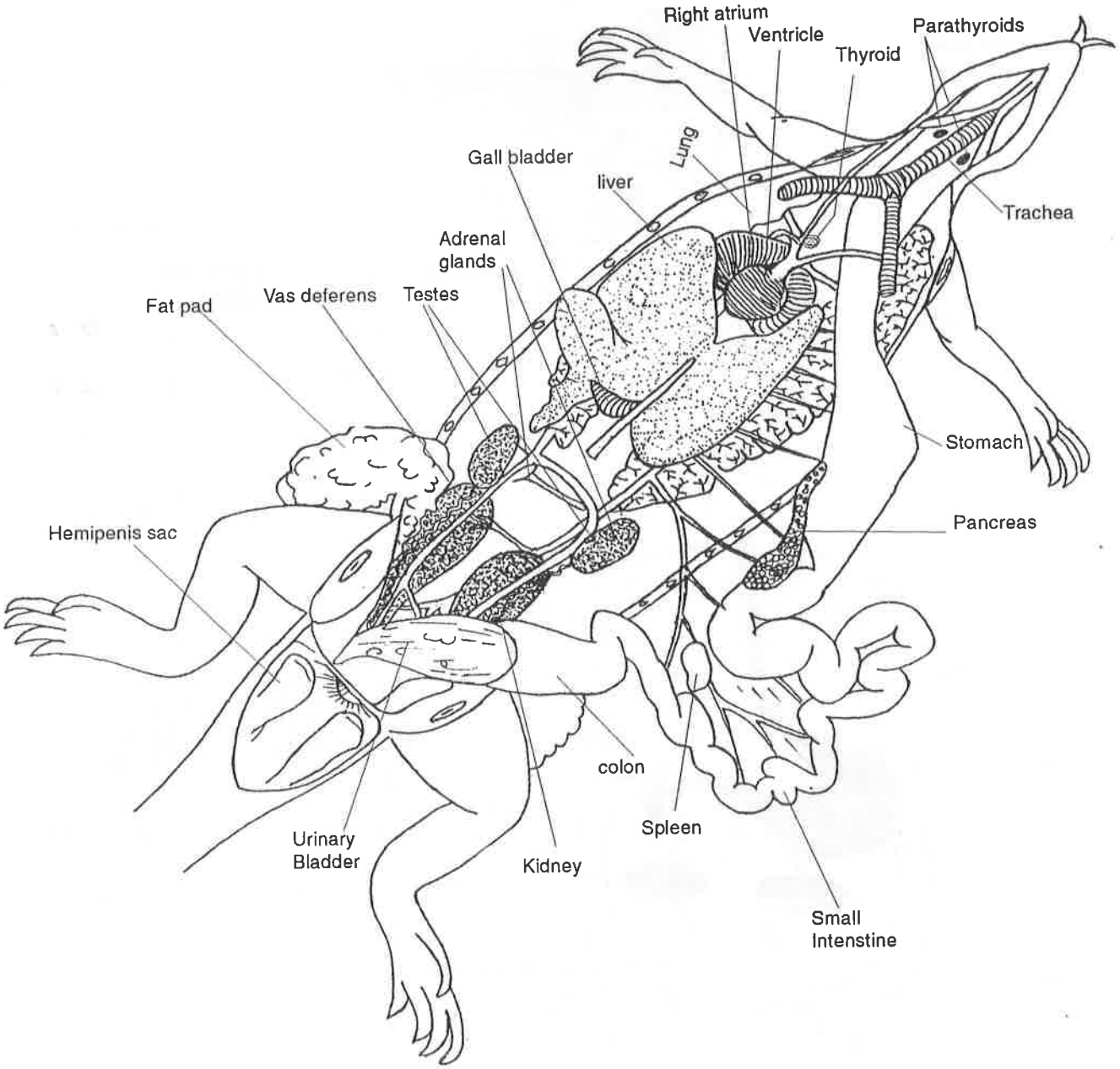


- Insert the blunt point of scissors on one angle of jaw to open the pharynx, oesophagus and crop.
- Lengthen the intestine by blunt dissection of mesentery (use scissors if necessary)
- Remove the pancreas from the duodenal loop by cutting its attachment with the duodenal loop.
- Cut open the major joints and expose the bones and the muscles.
- Examine the kidneys in situ.
- Open the proventriculus and gizzard with scissors and examine the contents and mucosa.
- Peel off the gizzard kaolin layer.
- Make a nick on the duodenum.
- Cut open the intestine to its full length.
- Cut open caecum.
- Make incision through vortex of skull longitudinally and transversely and reflect them outwards. Examine the meninges. Cut open the meninges to expose the brain. Scoop the brain out by cutting the ventral attachments and examine the brain.
- Expose the sciatic nerve in between the posterior two thigh muscles and examine.

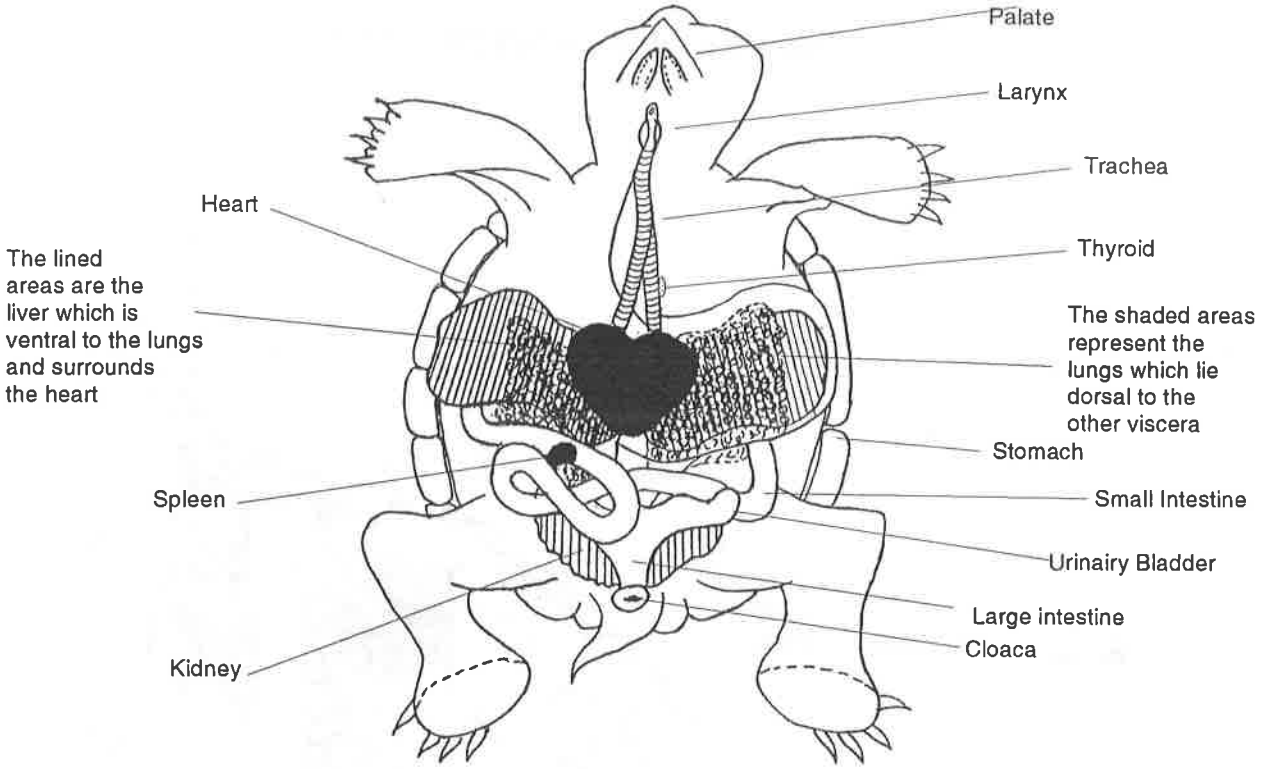
7. Reptiles

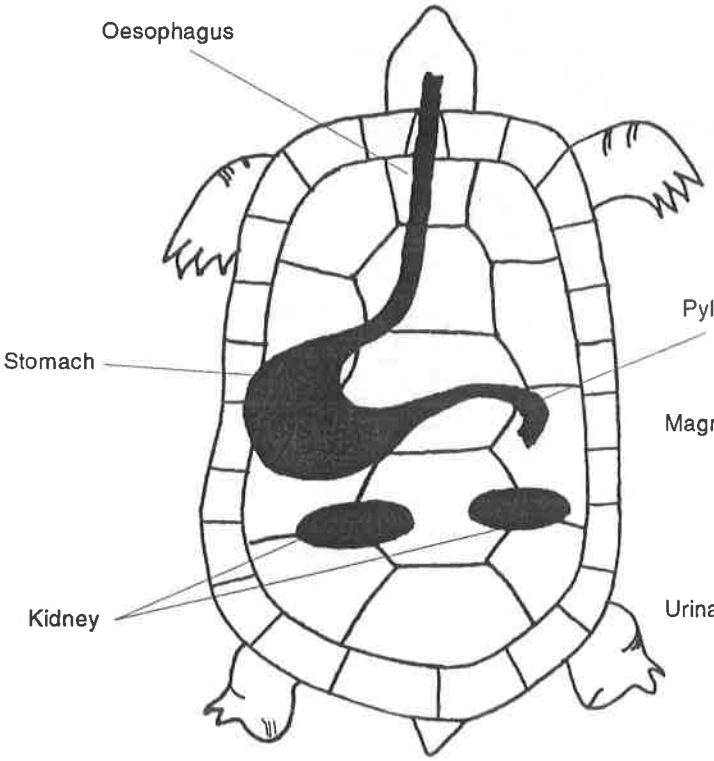
- Check external body surface and orifices for lumps, scales, ectoparasites, discoloration, wounds , discharges etc.
- Place the reptile on its back.
- For chelonians remove the plastron, by severing the body bridges between the plastron and the carapace and cutting through the muscular attachment.
- In non-chelonians , make a ventral midline incision from mandibular symphysis to cloaca.
- Reflect the skin laterally and pin them on the mounting board.
- Remove the coelomic fat to expose the viscera.
- Examine the viscera for abnormalities like position, adhesions etc.
- Incise the trachea and main stem bronchi and examine the lungs. (The left lung is vestigeal or absent in most snakes)
- Examine for lesions and parasites and sample for histology (Multifocal pigmented areas are normal in respiratory and liver tissue of reptiles particularly in diurnal lizard)
- Examine the heart epicardial surface (There is only a single ventricular chamber in all reptiles except for crocodilian and all reptiles possess two aortas) for chalky white deposits
- Examine the thymus (which is located anterior to the heart and extends into the neck in most species and usually paired and reduces in size with increasing age) for its size colour etc.
- Examine the thyroid located immediately anterior to the heart base in snakes or close to tracheal bifurcation in those with

VISCERA OF A MONITOR LIZARD

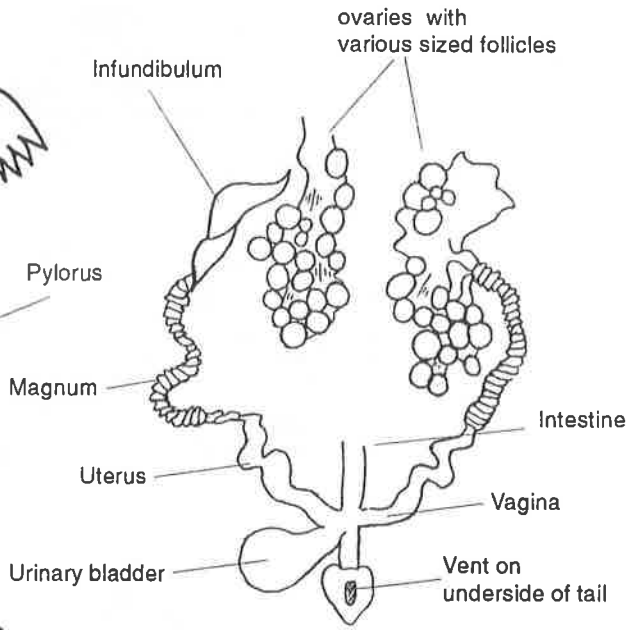


VISCERA OF CHELONION



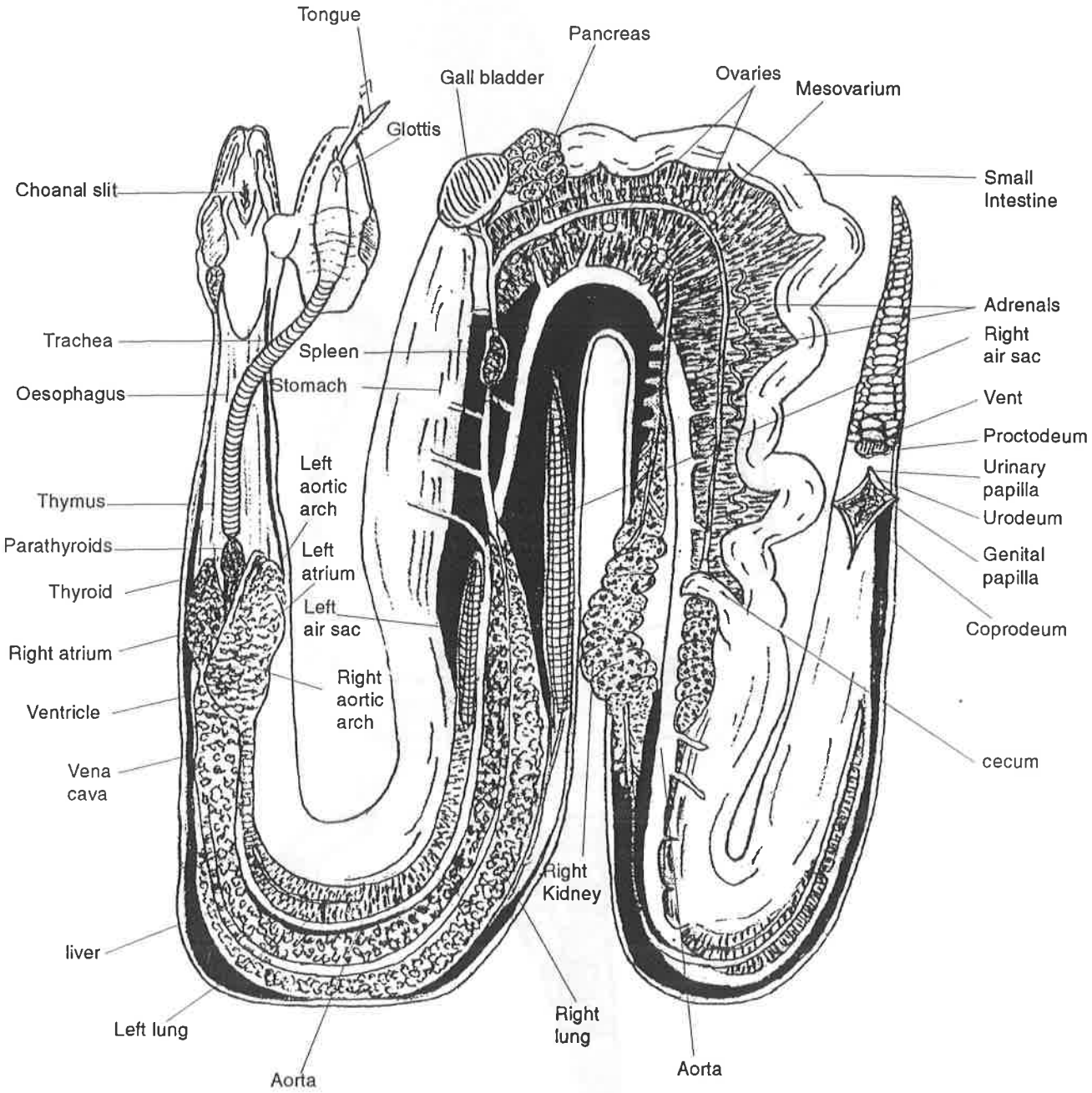


STOMACH & KIDNEY POSITION IN CHELONION

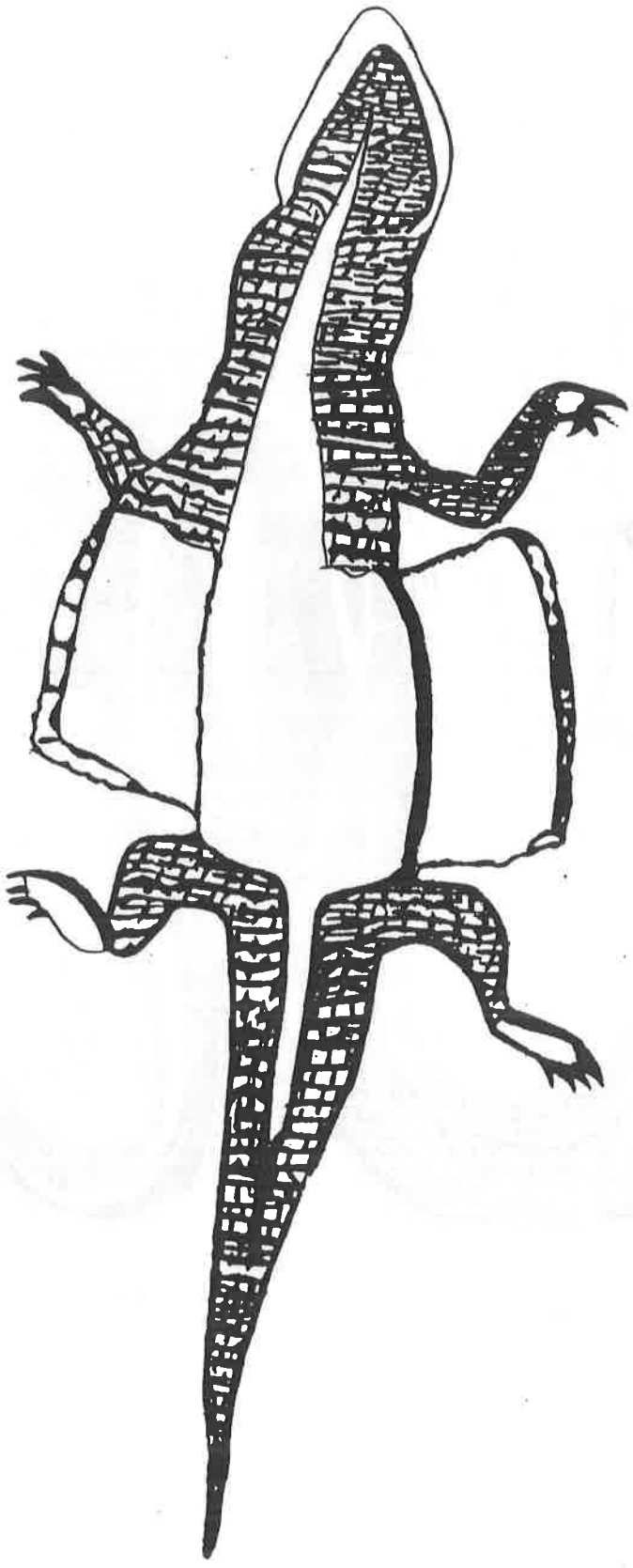


VENTRAL SURFACE OF GENITALIA FROM MATURE FEMALE

VISCERA OF A SNAKE



NECROPSY OF CROCODILE



paired lung and is single in most species and has glistening red colour.

- Cut the oesophagus behind the mouth and at the cloaca and remove the viscera enmass
- Open the digestive tract to its full length and check for lesions and parasites
- Scrape the mucosa of intestine with blunt edge of knife and collect scrapping on a clean microscopic slide for the presence of parasites.
- Examine the spleen located close to the stomach. (Spherical to triangular in snakes and crocodilians but elongated in most lizards)
- Examine the pancreas which lies adjacent to the duodenum and close to the spleen. (It varies in shape from spherical to cigar shape.)
- Examine the kidneys (which are paired and caudally located) for evidence of chalky white deposits . (In snakes the right one is anterior to the left and is flattened).
- Check the urinary bladder (Present only in chameleons and most lizards - and absent in snakes and crocodiles) for calculi.
- Examine the gonads. (The testis is usually elongated and yellow to white in color but pigmented in many chameleons. The ovaries have multiple follicles - bunch of grape appearance). Open the tubular genital tract (the oviduct is situated posterior to the ovary) and look for its contents, mucosa colour, thickness etc.

Make up to 1000ml with distilled water. Distribute in 20 ml amounts. Autoclave for 15min at 121 °C. Omit phenol red for fluorochrome detection of mycoplasmas.

For use

Stock solution A	100ml
Stock solution B	100ml
Distilled water	800ml

Dispense in 100ml amounts. Sterlize by autoclaving at 121 °C for 20min. Store at 4 °C.

4. Parasitology

The collection of parasites from a carcass should be carried out as systematically and completely as possible. For this, it is advisable to follow a definite scheme and examine each organ beginning from outside of the body then the cutaneous tissue spaces, the body cavities, internal organs, circulatory system, gastro intestinal tract and so forth. Prior knowledge on the various species of helminth present in the body is necessary.

a. Collect the parasites like ticks, mites, flees etc. from the surface of the body. While skinning the carcass, look for parasite from subcutaneous tissues, lymphnodes, muscle etc.

b. When examining the surface of the organs in the thoracic and abdominal cavity examine for worm which live freely or attached to organs as cysts.

c. Look for worms in the anterior chamber of eyes.

d. Examine separately the trachea, lungs, heart, oesophagus liver, kidney, urinary bladder etc. on both the surfaces the luminal and cut surface.

e. Collections from different organs must be kept separately and labelled.

f. Rinse the hollow organs and duct openings thoroughly with normal saline and examine the washings.

g. Tie off and separate the stomach, intestines and caecum.

h. Examine the contents of stomach, intestine and caecum by emptying into a bucket of water for parasites. The contents may be poured through a sieve to separate and count the parasites.

i. Examine the contents for both macroscopic as well as microscopic worms.

j. Scrape the mucosa of gastro-intestinal tract and examine for minute worms and larval stages.

k. Collect a sample of faeces from the rectum Examine for helminth eggs or protozoan cysts.

l. Flatten the cestodes with their scolices and trematode by placing the parasite between glass slides and securing the ends with threads or rubber bands with gentle pressure and then fix in 5% formalin or 40% alcohol.

a. Faecal examination (Microscopic examination of faeces)

1. Direct or simple smear method

A direct smear is made by mixing small quantity of faecal matter / dung with 2 or 3 drops of water on a clean microslide until a uniform suspension is formed. Then a cover slip is placed gently on to the material of the microslide and examined under low power magnification (x 100) of the microscope and then using the high power magnification (x 400) for the details. This method, though simple and quick, cannot detect light parasitic infection. A negative finding does not exclude parasitic infestation.

2. Concentration method

This technique involves the principle of concentration of the eggs in a given quantity of faecal matter / dung and is of great value in the routine diagnosis.

i. Sedimentation method

In this method, the eggs are sedimented to the bottom. A representative faecal material / dung (1 to 2 grams) is taken in a cup and throughly mixed with 15 ml of water with a glass rod. Then it is poured through a strainer into another cup to remove the coarse matter and debris. The strained material is gently mixed by rotating the cup and then immediately poured into a test tube and centrifuged at 1500 rpm for 2 - 3 minutes. The supernatant solution is poured off and a drop of sediment is placed on the microslide with subsequent placement of coverslip on it. It is then examined under low power magnification. All types of helminth ova can be studied. It is best suited for trematode ova which are operculated and sink to the bottom.

ii. Floatation method

Emulsifying fluid with higher specific gravity than that of the ova present is used to float the ova to the top column of liquid. Saturated solution of Nacl (sp.gr 1.28) or saturated solution of sodium sulphate (sp.gr. 1.40) or 32.5% zinc sulphate solution (Sp.gr.1.18) or saturated sugar solution (Sp.gr.1.25) can be used in this method. This technique consists of two methods.

Lane's method

A small quantity of faeces / dung (2g) is mixed in 15-20 ml of satured salt solution, and strained through a strainer. The strained emulsion is centrifuged in a test tube at 1000 rpm for 2 minutes. The floating ova at the

VI. FORENSIC ASPECTS OF POST MORTEM IN WILDLIFE

Sick animals in the wild is an extremely difficult task to detect, except during an epidemic. Very rarely one could see a dead wild animal in a proper condition for conducting a thorough postmortem examination. Many a times, carcasses of dead animals are difficult to find in a thick forest. The scavenger animals like Wolf, Jackals and Wild dogs devour the carcase and make it unfit for any detailed investigations. It is an official procedure that a Veterinarian should be called to conduct the Postmortem examination. The time lag between reporting of the death and the arrival of veterinarian should be reduced to the minimum. One should remember that diagnostic procedure based on mere clinical signs and postmortem examinations is no longer acceptable to the scientific arena. So one has to collect as many samples as possible for taking laboratory confirmation, with gross photographs of the carcass and lesions in organs.

COLLECTING THE BACKGROUND INFORMATION

Collecting the background information about the probable cause of death is an important part of the disease investigation procedure. The following hints should be considered :

1. Clinical signs of the animal before death (this can be gathered from those who had seen the animal during the illness).

2. The surroundings of the dead animal should thoroughly investigated for the presence of pug marks (foot prints of wild

carnivores), hoof marks, presence or absence of fecal materials around the animal, struggle signs, discharge of body fluids like blood, uterine discharge, urine, saliva etc.

PRECAUTIONS TO BE TAKEN BEFORE THE POSTMORTEM

The following points have to be borne in mind before one proceeds with the postmortem.

1. If the animal is suspected to have died due to poaching and poisoning, the case should be handled by the forest department. Any case of poisoning should associate the legal investigation.

2. Protective clothing is always advisable when zoonotic diseases are suspected. Wearing rubber gloves, face mask, aprons and gum boots are desirable.

3. Check nostrils, mouth, anus and genital orifices of the carcass for evidence of blood stained discharge which are the signs of anthrax. Elephants, rhinos, primates and carnivores can also die of anthrax. Do not open the carcass if anthrax is suspected.

4. From the human risk point of view, rabies is an important zoonotic disease.

5. Take a colour photograph of the animal before postmortem. Include also the surroundings of the animal in the photograph. If you consider that some of the extremely important externally

visible abnormalities are of significant features (blood discharge, gun-shot wound, arrow wound, bite markings, abrasions, prolapse of internal organs, foot and mouth lesions, loss of hair, ear sore, fracture, maggot wound, electrocution marks etc), take a close-up picture of the same.

6. As far as possible a rough estimate has to be made on how long the animal has been dead. This can be assessed by observing the following PM changes.

a. Rigor Mortis (RM) : While the presence of RM would suggest a recent death, absence would suggest that either RM has not set in or it has disappeared. This rule is generally applicable during summer. In winter, RM takes longer time to disappear.

b. Autolysis : When RM is absent, the age of the carcass can be assessed, once it is opened. Muscles show autolysis and in advanced cases, the tissues are often soft and pulpy.

c. Heart Blood : Presence of unclotted blood in the heart is an indication of recent death.

EXTERNAL EXAMINATION OF THE WILDLIFE CARCASS

1. Look for bite marks of the carnivores.

   If present, do not conclude that kills need not be autopsied. Bite marks and feeding signs by carnivores can follow subsequently to a natural death. Moreover, weak, and sick animals are more likely to be predated on by healthy and strong animals.

2. Look for external injuries, like evidence of fight among two males (signs of injuries by antlers, horns, gun shot wounds, arrow wounds and bruises).

3. Palpate the limbs, flex and extend them for evidence of fracture.

4. If muzzle, eye balls and other soft body parts are damaged by scavenger animals, make a note of it.

5. Look for ectoparasite on the skin surface.

6. Examine the body for rashes and extensive areas of rough and hard skin caused by mange mites.

   Look for warble which are caused by infestation by warble fly (*Hypoderma* spp.) larvae.

7. In mammals, examine mammary glands for evidence of lactation.

FORENSIC INVESTIGATION TECHNIQUES FOR WILDLIFE LAW ENFORCEMENT

Wildlife forensic - a new area of veterinary science, aids the wildlife conservation officer in solving crimes against wildlife resources. The illegal killing or taking of wildlife species specimens for commercial purposes has been a major problem throughout the world.

Enforcement of conservation regulation is an important tool in the management of wildlife resources.

An important function of the forensic pathologist is to document the manner, mechanism and cause of death. The causes of death may be an injury or disease that produce a physiological derangement in the body, which result in death. e.g. a gun shot wound to the chest. It may consider such factors as how far the animal traveled or how long it lived after being shot, since they may have direct application in providing the guilt or innocence of the accused. Hence as proof, the Vet should take a photograph of the gun shot wound if any, grossly of the carcass with the surrounding environment as well as a close up view of gun shot wound or the injury sites as proof later. In case of gun shot wound, the skin with the wound site should be taken and preserved for future as evidence in the court.

1. Gunshot Wounds

Recognition and description of gunshot wounds is a major component of wildlife forensic investigations. Gunshot wounds in animals covered with hair

or feathers are neither obvious nor as easily described as they are in a human body. Gunshot wounds in wildlife generally may be classified as high-velocity bullet wound and low velocity bullet wounds. Arrow wounds also must be differentiated from bullet or slug wounds. Wounds through the heart may not be immediately fatal; An animal may travel significant distance in the 10 to 30 sec. or longer it may take to drain blood from the circulatory system and for cerebral hypoxia to occur. The gunshot wound will show tearing and crushing of tissue along a wound path which is the result of the projectile striking the body and causing damage to the tissue. Some gunshot wounds produce significantly larger wound track as the result of tearing tissues and fragmentation of bones, which is non-expandable. Most gunshot wounds encountered in wildlife forensic cases are caused by relatively low velocity caliber guns.

Do not handle the bullets with forceps or other hard metal instruments. Carefully wash with water, dry, Wrap in gauze pad; Place each bullet or pellet recovered from the carcass in a separate paper envelop. Bullets that are of less penetrating velocity through the carcass are often found under the skin opposite to the side of the entrance wound. The bullet may not necessarily be found in close proximity to the haemorrhagic wound track. The exit hole or holes (more than one may be present from a single bullet) of a high-velocity bullet may be much larger than the entrance wound, and exhibit significantly more tissue damage. It also may have serrated edges or tissue strands, trailing to the exterior from the underlying subcutaneous tissue. Shotgun pellets have less velocity and has typically small tracks that penetrate vital organs and cause internal hemorrhage or fracture bones. The actual cause of death is

frequently trauma from the hunters bullet killing the small game, after it has been rendered immobile. Recovery of pellets is essential in determining the size and type of pellet used. Two or more sizes or types of pellets may be recovered from a carcass. However, this finding requires caution in interpretation as to whether two hunters are involved. Many hunters reload their own shotgun shells with wide variety of shot combinations.

2. Arrow Wounds

As archery hunting for big game animals has become more popular these days, the presence of both an entry and exit wound does not preclude the possibility that an animal was shot with an arrow.

Arrows from modern bows have the capability of completely passing through the chest of an animal of the size of spotted deer. Arrow points may be designed to be capable of fracturing large bones; Therefore, bone fractures are not always associated with bullet wounds.

3. Traumatic wounds

Vehicular or structural collision cases are commonly submitted for cause of death-determinations. Massive blunt trauma from a vehicle may obviously fracture bones and rupture internal organs. In other cases, only internal hemorrhages and subcutaneous bruising are evident. Skinning the carcass and examining the subcutaneous tissue for evidence of bruising is essential in evaluating suspected blunt trauma cases. The pattern of blunt trauma injuries

is very important to document, because it will provide information as to the instrument used to kill the animal.

Traps and snares often are used in the illegal killing of wildlife. Trapping wounds are seen mostly often on the neck (Snares), legs (usually leg-holds type of trap or snares on larger animals), or chest. Snares usually cause deep depressions in the skin, and hair is often broken. The flesh side of the hide often has reddened area where the snare was against the skin. A snare may collapse the trachea or damage the cartilages of the laryngeal area and cause death by asphyxiation. Subcutaneous hemorrhage is present in nearly all instances.

Trauma caused by predators and scavengers must be differentiated from other causes of trauma. Bite wounds from large predators such as wolves may produce more damage to underlying tissues than is evident by external observation. Scavengers and maggots frequently mask important lesions because they will start to scavenge a carcass at a location, such as a bullet or trauma wound, which is already open.

4. Electrocution wounds

Many wild animals submitted for cause of death determinations have been found with clues on contact with power lines or electrical fences or other source of electricity. However, the investigator should not assume that the victim died of electrocution.

The mechanism of death on most electrocution is ventricular fibrillation, which results in cardiac arrest. Intercostal muscle spasms may occasionally cause respiratory arrest. The only lesions observed internally are those of a congestive hypoxic death.

Occasionally, there may be fatal traumatic lesions associated with the violent spasms of electrically induced muscle contractions or a fall of high pole.

Evidence of electrical contact on an external body surface is not always present. When present, the lesion of a thermal-electrical burn has a typical appearance of a raised blister or focal burning of the epidermis and dermis. Feathers in case of birds associated with the site are typically curled. This should be differentiated from feathers that have been partially consumed by carrion eating animals and beetles.

Photographs of individual thermo-electrical lesions are always to be obtained.

5. Poisoned wildlife

The illegal use of poisons to kill wildlife is a major source of requests for the wildlife forensic specialist. Poisons commonly used to intentionally kill predators or others, include carbamate and organophosphate, pesticides, strychnine, anticoagulant rodenticide, thallium, and cyanide.

Wildlife enforcement officers have often observed animal hair at crime scenes or on clothing, pickups, trap, knives and so on as evidence. In addition, hair from the stomach of poisoned animals may be used to identify the source of the poison. Mammalian species have difference in the colouration, length, and texture of their coats. Different groups of mammals have different external and internal hair characteristics that are useful in identifying them to order, family, genus and species.

7. Time of death

Establishing the time of death is very important in wildlife forensic cases. Death has been defined as complete cessation of respiration and circulatory functions. Changes taking place after death may be helpful in assessing the time of death of the animal, although, post-mortem changes may be influenced by ambient temperature, health of the animal, degree of muscular activity before death, mode of death and medium in which the body has been kept etc.

The chief signs of death are (a) Cessation of respiration and heart beat, (b) Cooling of the body which starts after a few hours of death (8-12 hrs) which may depend on a number of factors, (c) Setting of rigormortis which is characterized by hardening and contraction of all voluntary muscles, rise in body temperature by about 3° F from normal temperature.

It starts from head and neck and later spreads to back and limbs. Maximum rigidity is attained after 20-24 hrs. and thereafter, it gradually

vanes out. Rigormortis occurs due to coagulation of myosin by lactic acid produced from the muscle glycogen in the absence of oxygen. It is related with the breakdown of ATP to provide energy for muscular contraction. Gradual disappearance of rigormortis is due to softening of the coagulated proteins by autolytic enzymes.

Muscular softening after the death may be primary flaccidity causing relaxation of the muscles, rigormortis causing stiffening of muscles and secondary flaccidity resulting into relaxation of muscles which is synchronous with the onset of putrefaction; Putrefication is the final change taking place in the resolution of the body. Factors responsible for putrefaction are warm temperature and air; Presence of air promotes and absence retards the putrefaction. Young animals decompose rapidly.

Putrefaction is retarded in poisoning (Arsenic, Antimony). The signs of putrefaction include distention of abdomen from gases, blood stained fluid from mouth and nostrils, liquefaction of eye balls, foul smell, bursting of abdomen and thorax and conversion of tissues to liquefied mass.

8. Forensic entomology

The time of death may be fixed within certain limits by the study of insects and their developmental stages within a decomposing carcass. This is a newly emerging science. It is based on the supposition that the invasion and destruction of a body of an animal - by necrophagous insects occur according to a predictable and sequential order that is dependent on environmental temperature. If accurate temperature and humidity records are available for

a area near where the carcass is found, the approximate time of death can be calculated by noting the developmental stages of the maggots (Diptera) and other insect species that inhabit the body. The pathologist or field investigator must use established techniques for the collection and preservation of mature insects or maggots from the carcass or from the crime scene for examination by a forensic entomologist.

VII. ZOONOTIC DISEASES OF WILD ANIMALS

The sanctuaries, zoological gardens and parks are the areas to encounter a wide variety of zoonotic diseases. Generally animals come to the zoos from wide varieties of geographic areas of the world after their capture, and they often spend weeks or months in close contact with the native people, which allows them to pick up human infections endemic to the locality. Added to this the "STRESS" of capture, captivity, drastic diet changes, transport and varying climatic zones through which the animal passes, sets the stage for these animals to shed pathogenic organisms communicable to human. In addition to these factors, the frequency of shedding of the contageons is also associated with over crowding of the animals.

Unlike the other places (animal and pet markets, infected sanctuaries etc.) zoos do not report a high incidence of zoonotic diseases. The possible reasons are as follows:

1. Zoo animals are usually not imported in great quantity in a single shipment.

2. Most zoo animals are procured through local animal dealers or other zoos rather than directly from the country of origin.

3. Because of high cost of species, zoos will not accept or pay for animals not in good condition of health.

4. Most zoos carefully check up animals after their arrival. This procedure includes Quarantine, a thorough veterinary

examination, tuberculin testing, serological screening for diseases, checkup for ecto and endoparasite, acclimatization, vaccinations and careful diet supervision before imports are added to the zoo collections.

1. DEFINITION OF ZOONOSES

Zoonoses are diseases- the agents of which are transmitted between vertebrate animals and man.

It is the interaction of agent, host and the environment they share that determines whether or not transmission of the agent will be successful, leading to infection and ultimately, occurrence of disease. Carrier host individuals infected without overt signs of diseases, are important in the persistence of many zoonotic agents. Vertebrate animals are the reservoirs of zoonoses. The agents may be transmitted either directly or indirectly by fomites or vectors.

It is estimated that there are over 175 infections and diseases of animals that are transmissible to humans under certain conditions. There are diseases common to man and animals inwhich they both generally acquire the infection from the same source. In certain cases, animals contribute in varying degrees to the distribution and actual transmission of infections. Lastly there are certain diseases inwhich humans are the primary hosts and animals are only infected when they are in contact with them.

Wild animals can transmit disease to humans in the following ways:

I. By Direct Skin Penetration

Anthrax

Leptospirosis

Meliodosis

Glanders

Tularemia

II. By Animal Bite

Rat bite fever

Herpes B.encephalomyelitis

Rabies

III. By Arthropod Vectors

Lyme disease	-	Tick
Plague	-	Flea
Relapsing fever	-	Tick
Tularemia	-	Tick and Biting flies
Rocky mountain spotted fever	-	Tick
Scrub typhus	-	Mite
Yellow fever	-	Mosquito
Encephalitis	-	Mosquito
Rift valley fever	-	Mosquito

2. PREVENTIVE STEPS IN WILDLIFE ZOONOSES

1. The capture of wild animals for trade or laboratories or for use as pets should be discouraged.

2. Quarantine procedure should be implemented for such animals.

3. Pets caught in wild or belonging to exotic species are dangerous because they may carry diseases that are zoonotic.

4. Wild animals should not be kept as pets.

5. Avoid infected animal tissues and premises contaminated by animal urine, blood or tissues.

6. People should wear protective clothing such as apron, rubber gloves, face mask when handling infected materials.

7. Swimming in fresh water ponds and streams likely to be contaminated by urine of wild animals should be discouraged.

8. Avoid contact with flies and ticks in enzootic areas during the seasonal incidence of biting arthropods.

3. CONTROL OF ZOONOTIC DISEASES IN WILD ANIMALS

The control of wildlife zoonoses must be approached in a multi disciplinary measures, because of the issues regarding the animal reservoir and mode of transmission of the contageon, the measures that must be taken

across human and veterinary medicine, sanitary engineers and in some cases the entomologist and wild life zoologist.

The measures of control includes:

1. Eradication of infected animal reservoirs/population reduction. There are many techniques of wildlife population reduction and selection of proper one depends on local conditions and regulations. Acceptable methods used are:

    a. Hormonal inhibitors
    b. Automatic vaccination devices to vaccinate selected wild animals
    c. Oral bait vaccination.

2. Protection of animals before they become infected : Improved sanitary measures and vaccination.

3. Eradication of biting arthropods : Use of insect repellents, insecticide application and sanitary measures.

VIII. A LIST OF ZOONOSES (SOME DISEASES NATURALLY TRANSMITTED BETWEEN VERTEBRATE ANIMALS AND MAN)

The following list of zoonoses is not comprehensive, although all the known major ones have been included. The diseases listed have been confined to those in which the animal link in the chain of infection to man is considered to be of importance, although not always essential.

Disease	*Causative Organism*	*Animals Principally Involved*
1. VIRAL DISEASES		
Arthropod-borne virus infections:		
Colorado tick fever	Colorado tick fever virus	Rodents
Eastern equine encephalitis	Eastern equine virus	Birds, equines
Encephalomyocarditis	Encephalomyocarditis virus	Rodents
Japanese B encephalitis	Japanese B virus	Birds, horses, swine and other mammals
Murray Valley encephalitis	Murray Valley virus	Birds
Rift Valley fever	Rift Valley fever virus	Birds
St. Louis encephalitis	St. Louis virus	Birds
Tick-borne spring-summer group (including louping-ill, Russian spring-summer encephalitis, Omsk haemorrhagic fever, Kyasanur forest disease)	Russian spring-summer-louping-ill group of viruses	Goats, sheep, birds, wild mammals
Venezuelan equine encephalitis	Venezuelan equine virus	Equines, rodents
Wesselsbron fever	Wesselsbron virus	Sheep

Disease	*Causative Organism*	*Animals Principally Involved*
Western equine encephalitis	Western equine virus	Birds, equines
West Nile fever	West Nile fever virus	Birds
Yellow fever (jungle)	Yellow fever virus	Monkeys
Aujeszky's disease (pseudorabies)	Aujeszky's virus	Ruminants, swine, dogs
B virus disease	B virus	Monkeys
Cat-scratch disease	Cat-scratch disease virus (?)	Cats
Cowpox (milker's nodule?)	Cowpox or vaccinia virus	Cattle
Equine infectious anaemia	Equine infectious anaemia virus	Equines
Foot-and-mouth disease	Foot-and-mouth disease virus	Ruminants, swine
Influenza	Influenza virus type A	Swine, horses
Lymphocytic choriomeningitis	Lymphocytic choriomeningitis virus	Mice, dogs, Monkeys
Newcastle disease	Newcastle disease virus	Chickens & Birds
Ovine pustular dermatitis (contagious ecthyma)	Ovine pustular dermatitis virus	Sheep, goats
Psittacosis (ornithosis)	Psittacosis virus	Psittacines, Poultry, Pigeons
Rabies	Rabies virus	Dogs, Cats, Wolves, Foxes, Jackals, Bats, Other wild animals
Sendai virus disease	Sendai virus	Swine, rodents
Vesicular stomatitis	Vesicular stomatitis virus	Equines, cattle, swine
2. RICKETTSIAL DISEASES		
Murine (endemic) typhus	*Rickettsia typhi (mooseri)*	Rats
North Queensland tick typhus	*Rickettsia australis*	Bandicoots, rodents

Disease	*Causative Organism*	*Animals Principally Involved*
Q fever	*Coxiella burnetii*	Cattle, sheep, goats, wild and domestic birds and mammals
Rickettsial pox	*Rickettsia akari*	Mice
Scrub typhus (Tsutsugamushi)	*Rickettsia tsutsugamushi*	Rodents
Spotted fever (including Rocky Mountain, Brazilian and Colombia spotted fevers)	*Rickettsia rickettsii*	Dogs, rodents and other animals
Boutonneuse fever Kenya thphus Also probably Indina tick typhus and South African tick-bite fever	*Rickettsia conorii*	Dogs, rodents
3. BACTERIAL DISEASES		
Anthrax	*Bacillus anthracis*	Ruminants, equines, swine
Brucellosis	*Brucella abortus, Br. suis, Br. melitensis*	Cattle, swine, goat, sheep, hares
Bacterial food poisoning and intoxications	*Salmonella spp., Staphylococcal enterotoxin, Clostridium welchii,* and others	Ruminants, swine, poultry, rodents
Colibacillosis	*Escherichia* spp., Arizona group of Enterobacteriaceae	Poultry, swine, dogs
Erysipeloid	*Erysipelothrix rhusiophathiae*	Swine, poultry, fish
Glanders	*Actinobacillus mallei*	Equines
Leptospirosis	*Leptospira* spp.	Rodents, dogs, swine, cattle
Listeriosis	*Listeria monocytogenes*	Rodents, sheep, cattle, swine
Melioidosis	*Pseudomonas pseudomallei*	Rodents, sheep, goats, equines, swine
Pasteurellosis	*Pasteurella multocida*	Mammals, birds

Disease	*Causative Organism*	*Animals Principally Involved*
Plague	*Pasteurella pestis*	Rodents
Pseudotuberculosis	*Pasteurella pseudotuberculosis*	Rodents, cats, fowls
Rat-bite fever	*Spirillum minus, Streptobacillus moniliformis*	Rodents
Relapsing fever (endemic)	*Borellia* spp.	Rodents
Salmonellosis	*Salmonella* spp.	Mammals, birds, poultry
Staphyloccoccosis	*Staphylococcus* spp.	Mammals
Streptococcosis	*Streptococcosis* spp.	Mammals
Tuberculosis	*Mycobacterium tuberculosis* var. *bovis* var. *hominis* var. *avium*	Cattle, goats, swine, cats, wild animals
	var. *hominis*	Dogs, swine, monkeys, wild animals
	var. *avium*	Poultry, swine, cattle, wild animals
Tularemia	*Pasteurella tularensis*	Rabbits, hares, sheep, wild rodents
Vibriosis	*Vibrio foetus*	Cattle, sheep
4. FUNGAL DISEASES		
Ringworm (favus)	*Microsporum* spp.	Dogs, cats, horses, wild animals
	Trichophyton spp.	Horses, cattle, poultry, small mammals
5. PROTOZOAL DISEASES		
Balantidiasis	*Balantidium coli*	Swine
Leishmaniasis :		
Espundia (American leishmaniasis)	*Leishmania braziliensis*	Dogs, cats, rodents
Kala-azar	*Leishmania donovani*	Dogs, cats, rodents
Oriental sore	*Leishmania tropica*	Dogs, cats, rodents
Toxoplasmosis	*Toxoplasma gondii*	Mammals, birds

Disease	*Causative Organism*	*Animals Principally Involved*
Trypanosomiasis: African sleeping sickness	*Trypanosoma gambiense (?)*	Wild and domestic ruminants
Chagas' disease	*Trypanosoma rhodesiense*	Wild games
	Trypanosoma cruzi	Cats, dogs, rodents
6. TREMATODE DISEASES		
Amphistomiasis (gastrodiscoidiasis)	*Gastrodiscoides hominis*	Swine
Bilharziasis	*Schistosoma japonicum* (and occasionally other species)	Ruminants, swine, dogs, cats
Clonorchiasis	*Clonorchis sinesis*	Dogs, cats, swine, wild mammals, fish
Dicrocoeliasis	*Dicrocoelium dendriticum*	Ruminants, equines
Echinostomiasis	*Echinostoma ilocanum* (and occasionally other species)	Cats, dogs, rodents
Fascioliasis	*Fasciolo hepatica, Fasciola gigantica*	Ruminants
Fasciolopsiasis	*Fasciolopsis buski*	Swine, dogs
Heterophyiasis	*Heterophyes heterophyes*	Cats, dogs, fish
Metagonimiasis	*Metagonimus yokogawal*	Cats, dogs, fish
Opisthorchiasis	*Opisthorchis felineus* (and occasionally other species)	Cats, dogs, wildlife, fish
Paragonimiasis	*Paragonimus westermani*	Cats, dogs, wildlife
Swimmer's itch	*Schistosoma* spp.	Birds, rodents
7. CESTODE DISEASES		
Diphyllobothriasis	*Diphyllobothrium latum*	Fish, carnivores
Diphylidiasis	*Diphylidium caninum*	Dogs, cats
Hydatidosis	*Echinococcus granulosus* (Larval cyst stage) and occasionally *E. multilocularis*	Dogs, ruminants, swine, foxes and rodents

Disease	*Causative Organism*	*Animals Principally Involved*
Hymenolepiasis	*Hymenolepsis nana*	Rats, mice
Sparganosis	*Sparganum mansonoides* (and other species)	Cats, mice and other mammals
Taeniasis and cysticercosis	*Taenia saginata* *Taenia solium*	Cattle Swine
8. NEMATODE DISEASES		
Ancylostomiasis and cutaneous larva migrans ("creeping eruption")	*Ancylostoma brazilliense* (and occasionally other species)	Dogs, cats
Strongyloidiasis	*Strongloides stercoralis*	Dogs
Toxocariasis (visceral larva migrans)	*Toxocara canis* *Toxocara cati*	Dogs Cats
Trichinosis	*Trichinella spiralis*	Swine, rodents, wild carnivores
Trichostrongyliasis	*Trichostronglyus colubriformis* (and occasionally other species)	Ruminants
9. ARTHROPOD AND INSECT INFESTATIONS		
Acariasis	*Dermanyssus* spp. *Sarcoptes* spp., *Trombicula* spp., etc.	Poultry, domestic animals, wildlife
Bug-bites	*Cimex* spp., *Triatoma* spp., etc.	Chickens, birds, small mammals
Flea-bites	*Xenopsylla, Ctenocephalus, Ceratophyllus, Tunga,* etc.	Rats, dogs, cats, swine, birds
Myasis	*Oestrus, Hypoderma, Gasterophilus, Cochliomyia,* etc.	Ruminants, equines
Tick-bites	*Ixodes, Dermacentor, Rhipicephalus, Haemaphysalis, Amblyomma, Argas,* etc.	Dogs, cattle

IX. DISINFECTION AND DISINFECTANTS

Among the measures recommended for the control of the disease, is the use of disinfectants, such as carbolic acid, chloride of lime and chlorine water, to destroy the infective agents. This view was expressed nearly twenty years before the bacteriological era can be said to have begun. At first phenol was the disinfectant of choice, but soon other coal-tar derivatives, in either a soluble or an emulsified form came into favour. The fifty years have seen a multiplication of new disinfectants, belonging to a series of different chemical groups, each with a limited and sometimes specific sphere of usefulness. The real foundations of modern system of disinfection and sterilization were firmly laid by number of investigators during the nineteenth century, particularly in the latter half.

Disinfection is the process of rendering a place, area (Stables, Animal covers) harmless by using physical or chemical agents to destroy the pathogens. Normally the term refers to the disinfection of one kind of germ only.

Disinfectants are agents that destroy the germs of putrefaction or disease, as well as the poisons liberated by these germs and also vectors, thus inhibiting the disease development. But one disinfectant need not necessarily affect all the germs, although it may be effective for certain pathogens.

Antiseptics are agents that seek to prevent infection by destroying germs in general before they gain entrance to tissues and places. It may be

accomplished by the use of flame, by means of steam under pressure or by means of immersion in boiling water for a definite period of time or with very powerful chemical agents.

The disinfectants exerts their action:

i. by oxidation
ii. by the removal of water
iii. by coagulation
iv. by chemical reaction

1. NON-CHEMICAL DISINFECTANTS

a. Sun Light

Sun light is deadly to the germs if it can reach them. This ability is due to ultra violet rays.

b. Heat

Heat is deadly to all forms of life including bacteria, virus, parasites, if applied with sufficient temperature. Blow lamping can be carried out for disinfecting animal house for the destruction of ticks and mites. Moist heat is also highly effective in the form of steam under pressure and scalding by hot water. The addition of 1% of "Salsoda" to the water will prevent the rusting of iron or steel during the disinfecting process. Steam disinfection shrink wood and destroy leather, rubber shoes and articles containing varnish and glues.

Autoclaving is considered as the best for destroying bacteria. Electricity by its heat generating ability or by its powers to free elements from chemical combination or by direct action, is destructive to germ life. Hot water has got the property of destroying the germs. But a temperature above 70°C is essential for effective destruction. This is commonly used for sterilizing steel equipments and glass vessels and syringes. The momentary application of boiling water to contaminated articles is not disinfection. But if such articles can be boiled for twenty minutes or so, then disinfection does good.

Steam may be used as saturated steam in super heated state. By saturated steam is meant the steam which is in such a condition that the heat full of temperature, condense to form vapour. It may be at different temperatures and the temperature depends upon the pressure under which it is generated. The greater the pressure the higher is the temperature.

2. CHEMICAL DISINFECTANTS AND RADIATIONS

Chemicals act as disinfectants by their oxidising power, their reducing power and their corrosive and coagulant effect on the albumin of bacteria and parasites. All disinfectant solutions should be made with clean water. The vessels in which the solution is made should also be clean. It is better to make the disinfecting solution with hot water than with the cold water and such solution is more powerful.

a. Perchloride of Mercury

It is in a powder form and frequently the powder is compressed into tablets. It is powerful disinfectant as solution of one part of disinfectant in solution of bichloride of mercury in a thousand part of water. One part of it in 500 parts of water will kill spores. For use on the mucous membranes in the mouth, eyes, genitals it is occasionally employed in solution, varying in strength from one part of it in 3000 to 5000 parts of water.

b. Bleaching Powder

It is known as "Chlorinated lime" which is a powerful disinfectant because of the large amount of chlorine gas incorporated in it. It is also an effective deodorant.

c. Lime

Chemically this is a oxide of lime (Quick lime). Quick lime mixed with water and it forms true hydrated or hydroxide of lime or milk lime. Good quality lime will produce a good deal of heat when mixed with water and this fresh product has a good disinfectant effect. For disinfecting premises contaminated with Foot and Mouth virus, a white wash made of 51bs slaked lime, one 1b of lye in 40 lts of hot water can be used as a spray to animal covers. Quick lime spread as powder has a good value as disinfectant on moist surface/dung pats and other animal discharges.

d. Formaldehyde

It is a good gas and when it is dissolved in water, the latter will take only 40% that is called as "Formalin". It is a very effective agent against T.B. organisms and Anthrax spores. For successful fumigation with formaldehyde gas, the room temperature must be above 65°F and there should not be any air current and the room should be tightly closed. Formalin with 10 parts of water is effective against all germs, viruses and spores if allowed to act for 2 hrs; About 300 ml of formalin is enough for fumigating 1000 sq.ft.

e. Coal tar

It is a disinfectant and antiseptic. But it is not much used for this purpose due to its strong odour and black colour, consequently they can only be used for disinfecting inanimate objects in the animal covers. They are reasonably effective against germs in the presence of organic material from the animal enclosures. One to two percent solution is strong enough to disinfect badly contaminated animal covers. Highly resistent spores are destroyed if permitted to act for atleast 24 hrs as 5% solution.

f. Phenol

The use of phenol as a disinfectant began with Lister. Phenol inactivates cells, irreversibly by disrupting the cell membrane, and causes cell lysis. Phenol and its derivatives are used in solution containing surfactants which improves its wetting properties. This enables phenolic disinfectants to be

adsorbed in thin and durable films on surfaces, resulting in surface disinfection. Their action is prolonged and lasts for many hours. Most common phenolic disinfectants are the bisphenols, cresol and hexachlorophene. Care must be taken while using phenolics, since these can damage the skin and the tissues.

g. Cresol

Basically the compound is derived from coal tar. The cresol is liquid at ordinary temperature. Saponated cresol solution (Lysol) is an emulsion of cresol made by the addition of raw linseed oil and lye. The product destroys most of the viruses and also acid fast Tuberculosis bacillus.

h. Lye

Chemically this is hydroxide of soda or hydroxide of potash or a mixture of the two. The product is noted highly as a disinfectant against most of the viruses and also against Anthrax spores. It is not effective against acid fast organisms which includes genus of Tuberculosis, Johnes disease and Gram positive organisms. The efficacy is more if used as cold rather than hot solution. For disinfection of wood work and animal enclosures, 2 lbs of lye in 45 lts of water is effective.

i. Potassium Permanganate

The product owes its germicidal action to the fact that in the presence of organic matter, it rapidly releases oxygen, which in a nascent state is very active. As a 4% solution, the product kills the spores of anthrax in 40 minutes. The organisms responsible for glanders in equdae is destroyed in few minutes. As a 1/1000, strength, it is very useful for washing the mucous membranes of the animal body.

j. Alcohols

The most commonly used alcohols are ethyl alcohol and propyl alcohol. These are mild disinfectants and are non-toxic when used for external application. Ethyl alcohol reduces the surface tension, dissolves lipid materials and also act as a coagulating agent in addition to being a dehydrating agent. Thus it is both a cleansing agent as well as mild disinfectant. Higher concentrations (95-100%) are less effective and generally a 70% solution is used.

k. Gases as Disinfectants - (Fumigation)

Fumigation is a means of disinfection by the use of the vapour of powerful antiseptic. This method of disinfection is not reliable by itself. But it may be used in conjunction with other forms of disinfectants. The difficulty is the impossibility of maintaining the gas in the same strength for a specific

period. In open buildings fumigation is useless. Sulphuric acid gas, chlorine and formalin are the three gases commonly used.

i. Sulphuric Acid Gas

This is generated by burning flower of sulphur or stick of sulphur in a room, the atmosphere of which is moist. To be effective 2 lbs of sulphur is used for 1000 C.ft. of space.

ii. Chlorine

Chlorine is generated by the action of sulphuric acid on chlorinated lime. About 3 lbs of chlorinated lime is used for 1000 C.ft. and about one lb of sulphuric acid is added.

iii. Formaline

Formaldehyde gas has been recommended for decontamination purpose. For this, special formalin tablets are used or one can liberate formaline lamp. In recent years, a Polymer of formaldehyde, namely Para-formaldehyde has also been used.

iv. Radiations

Sterilization by radiations is also referred to as cold sterilization. Radiations such as X-rays, gamma rays, ultra violet rays have been used as

sterilization sources for controlling microorganisms. The effectiveness of the radiation depends upon the degree of susceptibility of microorganisms.

3. DISINFECTANTS AND THE CONCENTRATIONS FOR USE

Sl. No.	Name of Disinfectant	Concentration	Use
1.	Ammonia	10%	Lethal to oocysts of coccidia.
2.	Calcium Oxide (quick lime)	--	To cover carcasses and contaminated premises.
3.	Cresol and Cresol with soap	5% 3 - 5%	Active in the presence of organic matter.
4.	Chlorinated lime (Bleaching powder)	3 - 12 ppm	Buildings and the surroundings.
5.	Cetrimide	0.5%	Disinfection of animal cages and hospital premises.
6.	Chlor hexidine	0.5%	Disinfection of animal cages and hospital premises.
7.	Formaldehyde	--	For fumigation of animal houses and disinfection of buildings as spray.
8.	Iodophors	1%	Cleaning equipments, contaminated vessels and trays.
9.	Phenol	2%	Active in the presence of organic matter.
10.	Potassium permanganate	1/1000 with formaldehyde (3:5) 45 to 90g of potassium permanganate for $3m^3$	Antiseptic fumigation for incubators and laboratories.
11.	Sodium hydroxide (NaOH)	2-4%	Disinfection of buildings esp. floors, penetrate contaminated organic matters. Effective against viruses.
12.	Sodium carbonate	4%	Contaminated area with foot and mouth viruses.
13.	Quaternary Ammonium Compound	0.1 to 0.5%	Effective on most bacteria and not effective on viruses and bacterial spores.

X. DISPOSAL OF CARCASSES OF WILD ANIMALS

The proper disposal of the carcasses of wild animals affected with contagious diseases and in particular, notifiable diseases is of the utmost importance in order to prevent the spread of disease and as in the case of anthrax to prevent human infection. Even after the death of animals from contagious diseases, their carcasses remain as reservoir of disease producing organisms.

PRECAUTIONS

1. Ensure that blood or discharges are not spilled upon the ground and this can be achieved by stuffing straw or grass saturated with some strong disinfectant into all the natural orifices like mouth, anus, vagina and ears etc.

2. Don't dispose the carcass by disposing it in or near a stream of flowing water, since this will carry infection to points downstream.

3. Animals dead due to this contagious disease if kept unattended, there is always the risk of healthy animals becoming directly or indirectly infected with the contagion.

The most sanitary method of disposal of carcass is to burn them. One method of burning a carcass is to dig a trench in the shape of a cross. Each trench is about 7 ft along about 15" wide and 18" deep at the centre. The earth is thrown into the angles formed by the trenches and on this earth is placed

with two stout pieces of iron rods. The trench provides the draft. Wood is placed on this and then put on the carcass and then some more wood round the carcass and a little dry grass and waste oil will set in. Care should be taken to see that the fire do not spread to the nearby bush.

BURIAL OF CARCASSES

The animal should be buried so as to have clear 6 ft above it and it should be covered with sufficient quantity of lime/bleaching powder/other disinfectants. The site chosen for the burial should be such that there is no possibility of contamination to any water source of river. In some cases, the endangered wild animal carcasses ought to be cut before, so as to make the skin defigured, as some poachers may come later and may dig it up for the skin and bones.

XI. PREPARATION OF SMEARS

1. Blood smear

(a) Place a drop of blood on a clean grease free glass slide.

(b) Place the slide on a level and smooth surface of a table is such a way that the drop is on your right side.

(c) Hold the spreader slide along its long edges and is brought towards the blood drop at an angle of 30° to 40° with the glass slide. The edge of the spreader slide must be smooth.

(d) Make the smooth edge of the spreader slide to touch the left end of the drop. Immediately blood drop will spread along the width of spreader slide.

(e) Push the spreader slide ahead towards the left end of the slide with quick but uniform motion and with a light but even pressure. The blood follows the spreader to form a film.

(f) Allow it to stand vertically for air drying

(g) Label it at one end of the slide

2. Impression smears

(a) Remove the blood and tissue from the surface of the lesion to be imprinted by blotting with a clean absorbent material.

(b) Touch the middle of the clean glass microscopic slide against the blotted surface of the tissue to be imprinted.

(c) Make multiple impressions if necessary.

3. **Smears of scrapings**

(a) Take the scrapings by holding a scalpel blade perpendicular to the lesion's cleaned and blotted surface and pulling the blade towards oneself several times.

(b) Transfer the material collected on the blade to the middle of a glass microscope slide

(c) Place another slide over the material

(d) Apply gentle digital pressure on the topside to spread the material.

(e) The slides are smoothly slided apart.

4. **Smears of swabs**

(a) Moisten the swab with a sterile isotonic solution like 0.9% NaCl.

(b) Swab the lesion with the moist sterile cotton swab.

(c) Gently roll the swab along the flat surface of a clean glass microscope slide.

5. **Smear from fluids**

(a) Place a small drop of the fluid on a glass slide about 1-1.5cm from the end.

(b) Slide another slide backward at a 30°-40° angle until it contacts the drop.

(c) When the fluid flows side ways along the crease between the slides, quickly and smoothly slide the second slide forward until the fluid has all drained away from the second slide.

(d) This makes a smear with a feathered edge.

6. Wet film preparation

(a) Puncture the site for a small drop of blood, with all aseptic precautions.

(b) Hold the cover slip by the edge between two fingers and touch it to the drop of blood.

(c) Invert the glass slide on it. Do not press. The blood will spread evenly due to the weight of the glass slide itself.

(d) Lift the glass slide along with the cover slip

(e) Apply only a little Vaseline or paraffin all round the edge of the cover slip to seal the capillary space. It prevents evaporation.

7. Smears from aspirates of solid masses

(a) Spray the aspirate onto the middle of a clean glass microscope slide.

(b) Keep the prepared slide on a flat, solid, horizontal surface.

(c) Pull another slide (Spreader slide) backward at a 45-degree angle to the first slide until it contacts about one-third of the aspirates.

(d) Slide the spreader slide smoothly and rapidly forward.

(e) Next, place the spreader slide horizontally over the back one-third of the aspirate at a right angle to the prep slide.

(f) Allow the weight of the spreader slide to spread the material, resisting the temptation to compress the slides manually.

(g) Keeping the spreader slide flat and horizontal, quickly and smoothly slide it across the prep slide.

XII. COMMONLY USED STAINING PROCEDURES

1. Wright's stain

Stain preparation

a) Place 0.3g of dry wright's stain powder in a mortar and overlay with 3ml of glycerol.

b) Rinse with 100ml of absolute methyl alcohol, added in small amounts and place in a dark container.

c) Mix with magnetic stirrer about 1 week, without heat.

d) Filter before use.

Staining procedure

a) cover the dried blood smear completely with Wright's stain and allow to stand for 1 to 3 min.

b) Add an equal amount of buffered distilled water or neutral water

c) Allow the mixture to stand for 3 to 5min

d) Float off the metallic scum with a stream of water from a wash bottle or from the tap.

e) Air dry the smear or blot gently with a blotting paper.

f) Examine the stained smears.

2. Giemsa stain

a) Fix the smear in absolute methyl alcohol for 3 minutes.

b) Dilute 2ml of commercial stock Giemsa stain solution with 8ml of distilled water.

c) Flood the diluted stain on the smear

d) Allow to stain for 5 minutes, then dry and examine.

3. Wright - Giemsa stain

Stain preparation

a) 300mg of wright's powder and 30mg of Giemsa stain powder are ground in a mortar with 100ml of absolute methyl alcohol.

b) Allow to stand for 24 to 48 hours before using.

c) Staining procedure is similar to that one used in wright's staining.

4. May - Grunwald - Giemsa stain

Requirements

a) May -Grunwald stain diluted with 1 or 2 parts of buffered distilled water (pH 6.8 - 7.2) - Sol.A.

b) Giemsa stain diluted with 9 parts of buffered distilled water - Sol.B.

Staining procedure

a) Fixing the smear by immersing in a jar of absolute methyl alcohol for 2 to 10 minutes.

b) Transfer the smear to sol . A and keep for 3-15mins.

c) Without washing transfer the smear to sol. B and leave for 7-30mins.

d) Differentiate in buffered distilled water for about 5 min.

e) Rinse, dry and examine.

Advantages:

- Production of better stained cytoplasmic granules.

Staining Reticulocytes

Vital Stains

a. New methylene blue - 0.5g
Potassium oxalate - 1.6g
Distilled water - 100ml

b. Brilliant Cresyl blue - 1 gm
0.85% sodium chloride - 100ml
Sodium citrate - 0.4g

Staining procedure

- Place equal quantities of blood and vital stain in a small test tube.
- Allow to stand for 5 to 20mins.
- Smear a drop of the mixture on a slide to make a thin film.
- Counter stain with one of the Romanowsky stains in the usual manner

Reticulum, Punctate nuclei, Nuclei of leukocytes and Thrombocytes stain blue.

5. LEISHMAN/LEISHMAN - GIEMSA STAINING

(blood smear, impression smear from organs)

1. Dissolve 0.15g of Leishman's powder in 100ml of acetone-free methyl alcohol. To this 30mg of Giemsa powder can be added and used for staining blood smears. Good for identifying parasites
2. Cover the unfixed smear with Lesihman's stain for 1 minute
3. Add double the quantity of slightly alkaline distilled water or phosphate buffer (pH 7.2-7.4) and allow for 15 minutes.
4. Wash in distilled water, dry and examine under oil immersion objective for anthrax bacilli /bipolars or stain the heat fixed smear with Loeffler's methylene blue for 3 minutes, wash dry and examine for anthrax bacilli and bipolars.

6. GRAMS STAIN

Solutions required

1. Crystal violet (4g in 20ml of 95% ethyl alcohol + 10ml of 1% ammonium oxalate).
2. Lugol's lodine (iodine 1g+ potassium lodide 2 g + distilled water 100ml.)
3. Absolute alcohol + acetone (70 parts + 30 parts)
4. 0.5 or 1g carbol fuchsin in 1L distilled water.

Method

1. Prepare thin smear, allow to dry and fix with heat.
2. Apply solution 1 for 2 mins. wash in water.
3. Replace with solution 2 for 1min. Wash in water.
4. Decolourise with solution 3, until the smear ceases to lose colour.
5. Wash with water.
6. Counter stain with solution 4 for 30 seconds.
7. Wash in water, blot dry and examine under oil immersion.

Result

Gram-positive organisms	Violet
Gram-negative organisms	Red

7. ZIEHL NEELSEN STAIN FOR Mycobacterium tuberculosis

Solutions required

1. Carbol fuchsin (basic fuchsin 1 g, absolute alcohol 10ml, 5% phenol in water 100 ml.)
2. Acid alcohol (conc.hydrochloric acid 2ml. Methylated spirit 98 ml.)
3. 0.5% Methylene blue.

Method

1. The organism is not necessarily destroyed by ordinary heat fixation. Prepare, dry and fix the smear in methanol in a safety cabinet, allow to dry and then remove from the cabinet, refix, with heat.

2. Flood the slide with solution 1 and heat (not boil), leave for 5 minutes and re-heat for a further 5 minutes.

3. Wash well in water.

4. Decolorise with solution 2 (several changes) for 15 minutes.

5. Wash in water.

6. Counterstain with solution 3 for 2 minutes.

7. Wash in water, blot dry and examine.

Result

Acid fast bacilli	Red
Background	Blue

The method is also suitable for staining M.johnei after simple heat fixation of the smear.

8. MODIFIED ZIEHL NEELSEN STAIN FOR Brucella abortus

Solutions required

1. Dilute 1 : 10 carbol fuchsin
2. 0.5% acetic acid
3. 1% methylene blue.

Method

1. Using a safety cabinet, prepare smears and fix in methanol.
2. Stain with solution 1 for 20 minutes.
3. Wash with water
4. Decolorise with solution 2 for not more than 30 seconds.
5. Wash with water
6. Counter stain with solution 3 for 20 seconds.

Results

Brucella organisms	Red
Background	Blue

The same method or a modification of it is suitable for staining Coxiella burnetti and Chlamydia psittaci.

9. LACTOPHENOL - COTTON BLUE (Mountant -stain for fungal cultures)

Solutions required

Phenol 20g., lactic acid 10ml, glycerol 40ml, distilled water 20ml and cotton blue 0.075 g.

METHOD

1. Place a drop of stain on a slide and in this gently apply a small portion of the culture using a mounting needle.
2. Place a No.1 coverslip on top, avoiding formation of air bubbles and remove excess stain round the edges of the coverslip.
3. If a permanent preparation is required, seal the edges with nail varnish.

10. OIL RED O METHOD FOR FAT

Cryostat sections post-fixed in calcium-formal; short fixed frozen sections.

Preparation of stain

The working solution is prepared an hour in advance by mixing three parts of a stock solution of Oil Red O (saturated in 99 per cent isopropanol) with two parts of 1 per cent dextrin (to eliminate dye precipitation) and filtering just before use.

Method

1. Dry sections onto slides and rinse in 60 percent isopropanol.
2. Stain for 15 minutes in Oil Red O.
3. Differentiate in 60 percent isopropanol until a delipidised control section appears colourless.
4. Wash in water and counter stain nuclei with Mayer's haemalum for three minutes.
5. Wash well in tap water.
6. Rinse in distilled water and mount in glycerine jelly.

Results

Unsaturated hydrophobic lipids that are insoluble in the dye bath, and mineral oils, are stained red. Some phospholipids appear pink.

11. Van GIESON'S STAIN FOR COLLAGEN

Solutions required

1. Weigert's Iron-haematoxylin

Solution A

Haematoxylin	:	5 g
95% ethtanol	:	500 ml
(It keeps for 3 years)		

Solution B

Ferric chloride (FeCl36H2O)	:	5.88 g
Water	:	495 ml
Concentrated hydrochloric acid	:	5 ml

(It keeps indefinitely)

Working solution: Mix equal volumes of A and B. Put A in the staining jar or tank first for more rapid mixing. The mixture should be made just before using, but can be kept for 10-14 days at 4 °C

2. Van Giesons solution

Add fuchsin	:	5.5g
Saturated aqueous		
Picric acid	:	500ml

Keeps indefinitely

3. Acidified water

Add 5ml glacial acetic acid to 1 litre of water (tap or distilled)

Procedure

1. Dewax and hydrate paraffin sections.
2. Stain in working solution of Weigert's haematoxylin for 5 minutes (10 minutes if the solution is more than 4-5 days old).
3. Wash in running tap water.

4. Stain in van Gieson's solution, 2-5minutes. The time is not critical.
5. Wash in two changes of acidified water.
6. Dehydrate rapidly in three changes of 100% ethanol. This step also differentiates the picric acid.
7. Clear in xylene and mount in a resinous medium.

Result

Nuclei - black or brown, collagen-red; cytoplasm (especially smooth and striated muscle), keratin and erythrocytes - yellow.

12. CONGO RED METHOD FOR AMYLOID

This method is suitable for paraffin sections of formaldehyde-fixed material. The differentiation in alkali removes most of the dye that is initially bound to collagen and cytoplasm.

Solutions required

A. Congo red solution

Congo red	1.0g
50% (v/v) ethanol	200ml (It keeps indefinitely)

B. Differentiating solution

Ethanol	160ml
Water	40ml
Potassium hydroxide	0.4g

Dissolve, then add water if necessary to bring the final volume to 200ml (It keeps for months but replace if it turns brown).

C. Nuclear stain

An Iron -haematoxylin or an alum-haematoxylin.

Procedure

1. Dewax and hydrate the sections.
2. Stain with Congo red (Solution A) for 1 to 5minutes, until sections are deep red.
3. Differentiate in Solution B, 2-10 seconds, then rinse in copious distilled water and examine. The only red colour remaining in the section should be in elastic fibres and laminae and in strongly acidophilic structures such as the cytoplasmic granules of eosinophils and Paneth cells. Amyloid deposits are also stained, but may be quite inconspicuous in the wet section. The differentiation may be repeated if necessary, and the staining may be repeated if differentiation has been excessive.
4. Counter stain nuclei with an iron- or alum-haemotoxylin.
5. Wash in water, dehydrate in acetone, clear in xylene and cover, using a resinous mounting medium.

Result

Amyloid deposits red. With polarizing microscopy, green dichroism in amyloid. This may reveal deposits that are hardly visible with ordinary illumination. Nuclei blue or black, according to counter stain.

13. PEARL'S PRUSSIAN BLUE METHOD FOR IRON

The fixative must not be acidic and must not contain chromium. Largest amounts of histochemically detectable iron were preserved by immersion of tissues for 24h in 6% aqueous formaldehyde containing 0.27M calcium chloride, with the pH adjusted to 4.0 by addition of 0.1M NaOH or 0.1M HCI. Alcoholic fixatives or buffered aqueous formaldehyde solutions were somewhat less satisfactory.

Solutions required

A. Acid ferrocyanide reagent

Potassium ferrocyanide	2.0g
Water	100ml
Dissolve and add	
Concentrated hydrochloric acid	2.0ml

Prepare just before using

B. Counter stain for nuclei

0.5% aqueous saffranine or neutral red.

Procedure

1. Dewax and hydrate paraffin sections.
2. Immerse in acid ferrocyanide reagent (solution A) for 30 min.
3. Wash in four changes of water
4. Counter stain nuclei (solution B) for 1 min.
5. Rinse briefly in water.
6. Dehydrate and differentiate counter stain in 95% and two changes or absolute alcohol.
7. Clear in xylene and mount in a resinous medium.

Result

Blue precipitate with Fe 3+ liberated from ferritin and haemosiderin. Nuclei pink or red. Haemoglobin is not stained.

14. VON KOSSA STAIN FOR CALCIUM

The von Kossa technique is often designated as a histochemical method for calcium, but it is really a method for phosphate and carbonate, the anions with which the metal is associated in normal and pathological calcified tissues. The sections are treated with silver nitrate. The calcium cations are replaced by silver.

The von Kossa procedure provides sharp and accurate localization of calcified material in tissues.

Solutions required

A. 1% aqueous silver nitrate (AgNO3)

B. 5% aqueous sodium thiosulphate (Na2S2O3.5H2O)

These can be used repeatedly until precipitates form in them.

C. Counter stain (0.5% aqueous satranine or neutral red)

Procedure

1. De-wax and hydrate paraffin sections.
2. Immerse in silver nitrate (Solution A) in bright sunlight or directly underneath a 100 W electric light bulb for 15 minutes.
3. Rinse in two changes of water
4. Immerse in sodium thiosulphate (Solution B) for 2 minutes.
5. Wash in three changes of water.
6. Counter stain nuclei (Solution C) for 1minute.
7. Rinse briefly in water.
8. Dehydrate (and differentiate counter stain) in 95% and two changes of absolute alcohol.
9. Clear in xylene and mount in a resinous medium.

Result

Sites of calcium (insoluble phosphates and carbonates) black. Nuclei pink or red.

15. BEST'S CARMINE METHOD FOR GLYCOGEN

Preparation of Solution

a. Carmine Stock solution:

Carmine	- 2 g
Potassium Carbonate	- 1g
Potassium chloride	- 5g
Distilled water	- 60ml

Boil gently for 5 minutes

Cool and add 20ml concentrated ammonia,

Filter and store in a dark container at 4° C

b. Carmine working solution

Stock solution	15ml
Concentrated ammonia	12.5ml
Methanol	12.5ml

c. Best's differentiator

Methanol	40ml
Ethanol	80ml
Distilled water	1000ml

Procedure

- Dewa test and positive control section.
- Stain the Nuclei well (eg. With an iron haematoxylin stain)
- Differentiate in acid alcohol, so that the background is clear
- Stain with carmine solution 5-15min.
- Wash well in Best's differentiator
- Rinse in fresh alcohol.
- Clear in xylol and mount as desired.

Results

Glycogen	-	deep red
Mucin,fibrin	-	Weak red
Nuclei	-	blue

16. GRIDLEY METHOD FOR FUNGI

Fixation	:	Any
Section	:	Paraffin

Solutions

a. Aldehyle Fuchsin solution

Basic fuchsin	-	1g
70% alcohol	-	200ml
Conc. Hcl	-	2ml
Paraldehyde	-	2ml

Allow to stand for 3 days at room temperature until the solution turns deep purple. Store at 40C and fitter before use.

b. Sulphurous acid rinse

10% aqueous potassium metabisulphite	- 7.5ml
0.1N hydrochloric acid	- 7.5ml
Distilled water	- 135ml

The solution should be prepared fresh before use.

Method

- Dewax sections in xylol. Hydrate through graded alcohols to water
- Place sections in 4 per cent chromic acid for 1 hour.'
- Wash in running tap water for 5 minutes, then rinse in distilled water
- Place in schiff's reagent for 15 minutes.
- Rinse in sulphuric acid rinse, 3 changes of 2 minutes each.
- Wash in running tap water for 15 minutes.
- Stain in aldehyde fuchsin solution for 30 min.
- Rinse in 95 per cent alcohol, then wash in running water for 5 minutes.
- Rinse in 50 per cent alcohol.
- Counter stain in saturated tartrazine in cellulose for 45 seconds.
- Dehydrate, clear and mount in DPX.

Result

Fungal hyphae	- Deep blue
Yeast and conidia	- Reddish purple.
Elastic tissue and mucin	- Blue
Back ground	- Yellow

17. PERIODIC ACID SCHIFF TECHNIQUE FOR GLYCOGEN

Preparation of solutions

a. Periodic acid solution

Periodic acid - 1g

Distilled water - 100ml

b. Schiff's reagent

- Dissolve 1g basic fuchsin in 200ml of boiling distilled water
- Allow the solution to cool to 50°c
- Add 2gm potassium metabisulphite with mixing.
- Cool to room temperature, then add 2 ml concentrate Hcl
- Mix, and allow to stand overnight in the dark.
- Add 0.2g activated charcoal and shake 1 -2 minutes
- Filter through a No.1 whatman paper, when the solution should be either clear or a pale yellow color.

First dip the slide in the water bath below the level of water at an angle of 45°C to the column of water and then-slowly reduce the angle and move the slides near to the section. Once the edges of the section touch the slide, take out the slide out of water column the section will automatically come on the slide. Label the slide with the black lead pencil in frosted part.

In case of several sectionings, slide no. should be identified by serial number (1,2,3) in form of superscript over the slide. Keep the slide vertically in slide tray or blot to the filter paper to remove excess water. Keep the slide on slide warming table (48 to 55°C) for 2 to 3 minutes. After the wax in the section gets melted take out the slide and kept at room temperature in the staining rack. Store the unstained slides in staining racks.

d. Staining, mounting, and labeling of slides

Haematoxylin and Eosin stain

Requirements: staining racks, coverslip, dissecting needles, forceps, scalpels etc. Filter paper, DPX, canada balsm, staining jar, Harris haematoxylin, eosin (yellow), xylene, alcohol (various graded), distilled water, ammonia water, slides with section, acid alcohol , slide trays, microscope.

Procedure for H&E staining

Sl.No.	Reagent	Timing (hours)
Deparaffinize the section		
I	Xylene I	2 min
II	Xylene II with agitation	"

Hydration			
III	Alcohol /IPA	100%	1min
IV	"	90%	1 min
V	"	80%	1 min
VI	"	70%	1 min
VII	Water		2 min
Staining			
VIII	Harris haematoxylin		5min
IX	Water		1 min
X	Acid alcohol quick dip		One dip
XI	Water quick rinse		1 min
XII	Ammonia water		3,4 dip
XIII	Water		1 min
Dehydration			
XIV	Alcohol /IPA	95%	1min
XV	Eosin stain		1min
Drain the staining solution			
XVI	Alcohol/IPA	70%	1min
XVII	Alcohol/IPA	80%	1min
XVIII	Alcohol/IPA	90%	1min
XIX	Alcohol/IPA	100%	1min
Wipe the excess alcohol on the slides			
Clearing			
XX	Xylene		2min

Labeling of the slides: The identification number specified on slides (with pencil) should be rewriten with marker pen. Necessary entries should be made in relevant format and logbooks of different equipment after their use.

XIV. SEROLOGICAL METHODS OF DIAGNOSIS OF DISEASES

1. AGAR GEL PRECIPITATION TEST (AGPT)

PRINCIPLE

Agar gel precipitation test (AGPT) is a qualitative / quantitative test to detect / estimate the presence of antigen / antibody. If we are looking for a virus (antigen) in a clinical sample, it is reacted with hyperimmune serum containing antibody. The antigen and antibody migrate in the gel and where ever they meet, they form a precipitation 'arc' or line, which is observed as white line in the gel.

MATERIALS REQUIRED

Canine parvovirus

Freund's adjuvants, Complete and Incomplete (Difco)

Phosphate buffered saline, 0.01M, pH 7.2

Agarose, SRL

Sodium chloride, AR

Sodium acetate, AR

Sodium azide

Acetic acid, AR

Merthiolate

Microscope slides

Whatman filter paper No.1

PROCEDURE

Agar gel precipitation test was carried out as per the method of Williams and Chase (1971) with some modifications (Ramadass, 1974).

1. Inject rabbits intramuscularly with 1 ml of canine parvovirus vaccine, incorporated in Freund's complete adjuvant.

2. Give totally three injections at fortnightly intervals. Freund's incomplete adjuvant was used for second and subsequent injections.

3. Seven days after the last injection, test bleed the rabbits from the ear vein and separate the serum and test for antibody level by AGPT.

4. If sufficient concentration of antibodies are detected, bleed rabbits intracardially.

5. After separation of serum, store the serum at -20°C after addition of merthiolate to a final concentration of 1:10,000.

6. Faecal swabs taken in PBS (0.01 M and pH 7.2) is used as sample for viral detection.

7. Use a good quality agar (Agarose, SRL) and prepare a 1.2% agar in saline solution, boil and cool to 60°C and add sodium azide to a final concentration of 0.01%

8. Pour 4 ml of molten agar into each clean microscopic slide and allow it to solidify.

9. When solidified, keep in 4°C for one hr for complete setting.

10. Punch out the antigen and antisera wells and seal the bottom of the wells with little of molten agar to prevent leakage of antigen or antibody.

11. Charge the wells with 2 drops of the test materials and antisera in separate wells.

12. Incubate the slides in a moist chamber at room temperature for about 24 hrs.

13. After completion of the antigen-antibody reaction, the precipitation can be seen between sample and hyperimmune serum wells. The intensity of the precipitation arc is proportional to the concentration of the virus in the sample.

14. Wash the gel with 1.5% sodium chloride solution for about 2-3 days, changing the solution three times a day, to remove the soluble non-reacting constituents.

15. Dry the gel by placing a sheet of filter paper over the surface of the gel plate and keep at 37°C for 24 hrs.

16. After complete drying, remove the filter paper and clean the slides for few seconds in running water to remove the adhering filter paper particles.

17. Prepare the Amido Black stain as follows:

Amido Black stain	1 g
Acetic acid, 1M	425 ml
Sodium acetate, 0.1 M	425 ml
Glycerol	150 ml

18. Immerse the dried slides in the above stain for about 10 minutes.

19. Destain the slides using, 7% acetic acid until the gel background is decolourised.

20. When complete decoloration is obtained, dry the slides in air and record the results.

APPLICATIONS

AGPT is commonly used for the detection of antigens, either virus or bacteria in clinical samples. Diseases like canine distemper, canine parvovirus, rotavirus, adenovirus and other diseases could be detected using this technique. Occasionally, this test could also be used as quantitative test to detect antibody or antigen.

2. COUNTER IMMUNOELECTROPHORESIS (CIEP)

PRINCIPLE

Similar to AGPT, CIEP also involves antigen and antibody reaction. However, in this technique, the rate of reaction is hastened by passing current to move the antigen and antibody. Thus, the result is obtained faster than AGPT and sensitivity is also higher.

MATERIALS

Agarose, SRL

Barbituric acid, AR

Sodium barbitone, AR

Conjunctival swabs from suspected animals for canine distemper

Rabbit anti-distemper hyperimmune serum

Whatman filter paper No.1

Sodium chloride, AR

Sodium acetate, AR

Microscope slides

Electrophoresis apparatus

Amido Black stain

PROCEDURE

The counter immunoelectrophoresis was carried as per the methods of Diaz and Myers (1981) with some modifications (Ramadass et al., 1983).

1. Prepare Veronal buffer as follows:

Sodium barbital, AR	10.31 gm
Barbituric acid, AR	1.84 gm
Sodium acetate, AR	6.80 gm
Dist. Water to	1 L
Ionic strength 0.1 M	

2. Prepare the agar gel plates with 1.2% agar in veronal buffer as described earlier under the section AGPT.

3. After complete setting of the agar, punch out the wells (4 pairs of wells could be made in each microscope slide) 3-4 mm apart.

4. Place the suspected samples (antigens) on cathode side well and place the rabbit hyperimmune serum raised against canine distemper in the well in anode side.

5. Place the slide in the electrophoresis tank with contact wicks.

6. Run the electrophoresis at 150 V for three hrs. Duration of electrophoresis is to be standardized for each laboratory and different antigen detection.

7. By end of electrophoresis, observe for precipitation lines. For obtaining stronger reactions, incubate the slides at room temperature in moist chamber for 24 hrs.

8. Wash, dry and stain the slides as described under AGPT section.

APPLICATIONS

CIEP is commonly used for detection of antigens like bacteria and virus from clinical samples. Frequently, used in diagnosis of Rabies, Rinderpest, Distemper and Parvovirus.

3. HAEMAGGLUTINATION TEST (HAT)

PRINCIPLE

Canine parvovirus has the property of haemagglutinating erythrocytes of pigs, rhesus monkey and horse under restricted pH and temperature conditions. This property has been taken advantage in detection of canine parvovirus in faecal samples.

REAGENTS REQUIRED

Phosphate bufferred saline, pH 7.2

Sodium phosphate-sodium chloride buffer, pH 6.4

Pig RBC

U-Bottom 96-well plate

PROCEDURE

Haemagglutination test for detection of canine parvovirus is carried out as per the method of Senda *et al.*, (1986) with some modifications.

1. Serial two fold dilutions of the faecal samples starting from an initial 1:8 dilution is made in PBS.

2. Pig erythrocyte suspension (0.75% in sodium phosphate-sodium chloride buffer), 0.05 ml is added to each of the wells and gently mixed.

3. Positive and negative controls are included in the plate.

4. The plate is then incubated at 4°C overnight.

5. The HA titre is expressed as the reciprocal of the highest dilution showing complete HA.

6. HA is considered positive when the HA titre is more than 64.

APPLICATIONS

Haemagglutination test has been used for detection of canine parvovirus, Newcastle disease virus of birds and canine distemper virus.

4. FLUORESCENT ANTIBODY TEST (FAT)

PRINCIPLE

This method also involves antigen and antibody reaction. However, here we use antibody attached to a fluorescein dye to observe the antigen-antibody reaction. A smear of virus or any other microbe is taken in a glass slide, fixed and reacted with fluorescein isothiocyanate-antibody conjugate. In positive cases, the virus takes up the fluorescein dye which when examined under a fluorescent microscope, shows greenish yellow fluorescence. In direct immunofluorescence, specific antibody-FITC conjugate is used, whereas in

indirect method, the sample is treated with a primary antibody, which is subsequently reacted with a secondary antibody-FITC conjugate.

MATERIALS REQUIRED

Goat anti-rabbit IgG FITC conjugate (Sigma)

Microscope slide

Glycerol saline

Fluorescent microscope

PROCEDURE

Indirect method of immunofluorescence is used to demonstrate the canine distemper viral antigen in the conjunctival smears. This technique was based on the method of Johnson (1977).

1. Two conjunctival smears are taken on either side of glass slide from animals suspected for distemper. These smears are fixed in cold acetone for 1 hr at 4°C and then air dried.

2. The rabbit anti-distemper serum (2-3 drops) is added to the smear on the right hand side of slide and the normal rabbit serum is applied on the smear on the left hand side to serve as a control.

3. The slides are incubated at 37°C for 30 minutes in a humid chamber.

4. The slides are then washed in PBS 0.01 M pH 7.2 for about 10 minutes and the washing was repeated three times.

5. Then, the smears are dried and 2-3 drops of goat anti-rabbit IgG FITC conjugate (Sigma) 1:20 dilution are added and once again incubated at 37°C for 30 minutes in a humid chamber.

6. Slides are washed in PBS for 10 minutes; Washing is to be repeated three times.

7. Slides are dried and mounted with a cover slip over a solution of glycerol saline (1:1). These are then examined with a fluorescent microscope equipped with a HBO 200 Mercury lamp. The control smears are examined first, followed by test smears. In positive smears, granular specific fluorescence is observed in the cytoplasm of epithelial cells.

APPLICATIONS

FAT can be used for detection of canine distemper virus, canine parvovirus, rabies virus and FMD virus in clinical samples.

5. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

PRINCIPLE

In this technique, the antigen or antibody is attached to a solid phase. Then the test sample is applied to the solid phase. The antigen-antibody complex can be visualized by treating with antibody enzyme (horse radish

peroxidase) conjugate and a substrate. The colour developed is proportional to the concentration of antigen/antibody. The solid support can be either polystyrene microwell plate or nitrocellulose membrane as used in dot/dipstick ELISA.

The methods recommended by Thierman and Garrett (1983), Tabatabai and Deyoe (1984) and Cargill *et al.*, (1985) were used with few modifications.

MATERIALS AND REAGENTS REQUIRED

Phosphate buffered saline (PBS), pH 7.2

Tween 20

Carbonate-bicarbonate buffer, pH 9.6

Sodium citrate buffer, 0.05 M, pH 4.2

Hydrofluoric acid, 0.1 M

Substrate - 2',2-Azino-di-ethyl benzthiozoline-6-sulphonilic acid (ABTS; Sigma)

Heat killed letospira antigen.

PROCEDURE

IgG-ELISA is performed as per the method of Adler et al. (1981).

1. One hundred µl of antigen is diluted in 19.9 ml of carbonate-bicarbonate buffer (pH 9.6) and used for coating the microtitre plate at 4°C overnight.

2. After 3 washings with PBST, uncoated sites are blocked with 2% BSA solution with one hour incubation at 37°C.

3. Plate is washed as before and serially diluted serum was added. Plate is incubated at 37°C for 1 hr and washed in PBST.

4. Rabbit anti-human IgG-HRP conjugate is diluted (1:5000) and added to the wells.

5. Plate is washed after incubation for 1 hr at 37°C.

6. Substrate, ABTS is used at 5.5 mg in 25 ml of sodium citrate buffer. Twenty five µl of hydrogen perioxide is added to the substrate solution, which is added to the microtitre plate.

7. The plate is left at room temperature for colour development (5-10 minutes) and OD is measured at 405 nm in an ELISA reader. Well A1, substrate control is used to blank the reading. Any OD value, which is twice the negative sample OD is taken as positive OD value. The bluish green colour which is seen when ABTS is used is proportional to the concentration of the antibody in the serum sample.

APPLICATIONS

Plate ELISA technique is commonly used for quantitation of antibodies in serum samples. It can also be used for antigen detection. Antibodies against Rabies, Leptospirosis, Tuberculosis, Brucellosis, Foot and mouth disease,

Rinderpest, Theileriasis, Trypanosomiasis, Hydatidosis, Anaplasmosis and various other diseases have been estimated using plate ELISA.

6. DOT-ELISA

PRINCIPLE

Dot ELISA technique is usually used for detection of antigen from clinical samples. The main difference between dot ELISA and dipstick ELISA is that in the former, the reaction is observed on nitrocellulose membrane in the form of a brown dot, whereas in the case of dipstick ELISA the colour change occurs in solution which is the indication of positivity.

MATERIALS REQUIRED

Nitrocellulose membrane

Rabbit anti-distemper hyperimmune serum

Goat anti-rabbit IgG-HRP conjugate

Dried milk powder

Diamino benzidine

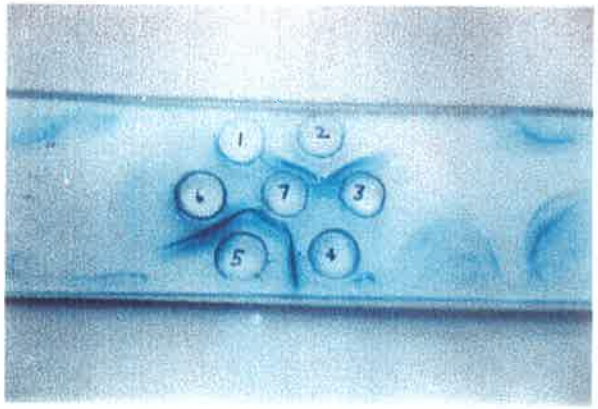
Phosphate buffered saline, 0.1 M, pH 7.2

PROCEDURE

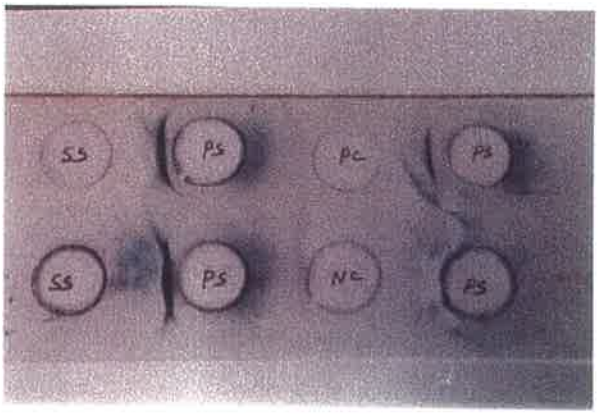
The test was performed according to the method of Ramadass *et al.*, (1996) with some modifications.

1. Conjunctival swabs are collected in PBS from dogs suspected for canine distemper.

AGAR GEL DIFFUSION TEST



COUNTER IMMUNOELECTROPHORESIS



HAEMAGGLUTINATION TEST

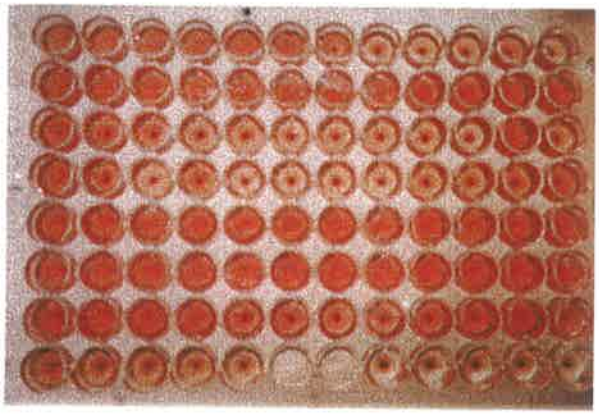
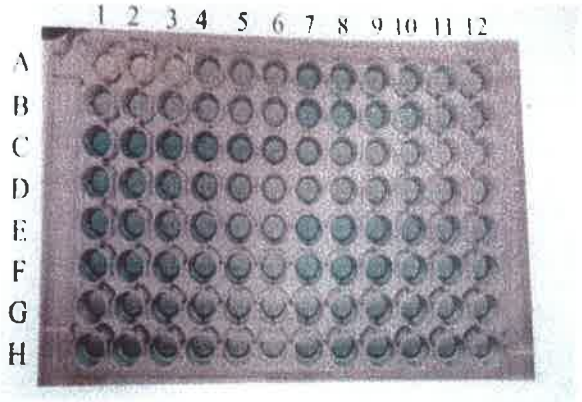
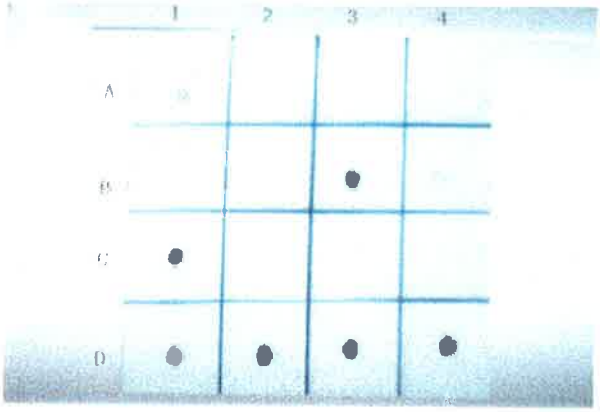


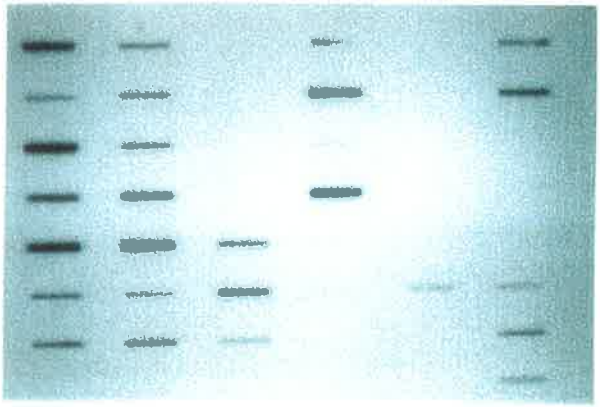
PLATE - ELISA TECHNIQUE



DOT - ELISA TECHNIQUE



SLOT - BLOT HYBRIDIZATION TEST



2. One µl of test samples is applied over a nitrocellulose membrane (NCM, Sigma) to cover one square mm area and air dried at 37°C for 10 minutes.

3. The NCM is treated with 0.06% hydrogen perioxide for one minute at room temperature to inactivate endogenous peroxidase.

4. The untreated sites are blocked with 5% skimmed milk powder in PBS containing 0.05% Tween 20 (PBST) for 10 minutes at 37°C.

5. At the end of incubation, the NCM is washed twice in PBST, 2 minutes each wash.

6. NCM is then incubated with rabbit anti-CDV hyperimmune serum in 1:100 dilution in PBST for 10 minutes at 37°C.

7. NCM is washed twice as before with PBST and treated with goat anti-rabbit IgG HRP conjugate at a concentration of 1:3000 and incubated for 10 minutes at 37°C.

8. NCM is washed twice in PBST and diamino benzidine tetrahydrochloride (10 mg in 10 ml of PBST) is used as a substrate along with 25 µl of hydrogen perioxide to bring out the colour reaction. The NCM is immersed in substrate solution for 4-5 minutes, until the brown colour reaction is seen. Once the colour develops, the substrate solution is discarded and NCM is washed in distilled water and air dried. The positive reactions are

2. Chloroform (300 µl) was added and mixed well and kept on ice for further 10 minutes.

3. The solution is then centrifuged at 10,000 rpm for 15 minutes at 4°C.

4. The supernatant (around 750 µl) is transferred to a clean 1.5 ml eppendorf tube.

5. An equal volume of isopropyl alcohol (750 µl) added to the above tube and mixed well. It is left on ice for 10 minutes.

6. The RNA is pelleted by centrifugation at 10,000 rpm for 10 minutes at 4°C.

7. Supernatant is discarded and the RNA pellet is washed with 1 ml of 75% ethanol by centrifugation at 10,000 rpm for 10 minutes at 4°C.

8. The RNA pellet is air dried for about 10 minutes and resuspended in 20 µl of DEPC water.

9. The RNA solution is incubated at 37°C for 30 minutes for complete resuspension and stored at -20°C until use.

RT-PCR reaction (Frisk *et al.,* 1999).

All the components, reaction mixes and samples are kept on ice. All the following reagents are added to nuclease free microfuge tube placed on ice.

10x Reaction buffer		5 µl
50 mM $MgCl_2$		1.5 µl
dNTP mix (10 mM each)		1 µl
Template RNA		10 µl
Primer 1		1 µl
Primer 2		1 µl
AMV RT		1 µl
DyNAzyme EXT DNA polymerase		2 µl
Sterile DEPC water	to	50 µl

The components are mixed gently and spin briefly. Depending on the thermal cycler used, overlay with mineral oil if required. A typical temperature profile is given below:

1. cDNA synthesis, inactivation of AMV Reverse transacriptase and denaturation of the cDNA/RNA hybrid

1 cycle of	42°C for 60 minutes
	75°C for 10 minutes
	94°C for 2 minutes

2. PCR amplification

Step 1 : Initial denaturation	94°C for 1 minute .
Step 2 : Denaturation	94°C for 1 minutes
Annealing	59.5°C for 2 minutes
Elongation	72°C for 1 minute 30 cycles

3. Final Extension 72°C for 5 minutes

APPLICATIONS

RT-PCR technique is used for detection of RNA viruses from clinical samples. This method could be used for detection of canine distemper virus and rabies virus from clinical samples.

c. NUCLEIC ACID PROBE PREPARATION AND HYBRIDIZATION PRINCIPLE

The development of nucleic acid probe is to identify nucleotide sequences which are unique to a particular organism of interest. The nucleic acid which contains such sequences is isolated, cleaned and labeled with a reporter molecule such as radioactive (^{32}p or ^{35}s) or non-radioactive (biotin, digoxigenin) molecules. The labeled DNA in single-stranded form is then hybridized to single-stranded nucleic acid in tissues (*in situ* hybridization), in paper (Southern blot) or in solution. If the nucleotide sequences in the nucleotide probes are complementary to those in the sample, hybridization occurs and results in the formation of double-stranded nucleic acid. Non-hybridized single-stranded probe is removed. Hybridization is monitored by autoradiography in the case of probes labeled with radioactive materials or calorimetrically or visually for probes with non-radioactive material.

MATERIALS REQUIRED

20x SSC	Sodium citrate	0.3 M
	Sodium chloride	3 M
	PH 7.0	

Primary Wash buffer containing urea

Urea	6 M	360 gm
SDS	0.4%	4 gm
20x SSC buffer		25 ml

Make upto 1 litre. This can be kept for upto 3 months in a refrigerator at 2-8°C. One hundred ml of 20x SSC is made upto 1 litre and can be stored at 2-8°C for 3 months.

Enhanced chemiluminescence (ECL) labelling and detection kit (Amersham)

- Saran wrap
- Hybridization oven
- Probe DNA
- Autoradiographic cassettes
- X-ray film

PROCEDURE

Preparation of labeled probes

1. Dilute the DNA to be labeled to a concentration of 10 ng/ml using the water supplied in the kit.

2. Denature 100 ng of the DNA sample (10 µl) by heating for 5 minutes in a boiling water bath.

3. Immediately cool the DNA on ice for 5 minutes Spin briefly in a microcentrifuge to collect the contents at the bottom of the tube.

4. Add an equal volume of DNA labeling reagent (10 µl). Add glutaraldehyde equivalent to that of labeling reagent. Mix thoroughly and spin briefly in a microcentrifuge to collect the contents at the bottom of the tube.

5. Incubate for 10 minutes at 37°C (when labeling nucleic acids less than 300 base pairs increasing the incubation period to 20 minutes may improve labeling efficiency).

6. If not used immediately, the probe can be held on ice for a short period, for example 10-15 minutes

Hybridization and stringency washes

1. Warm hybridization buffer to 42°C.

2. Prewet the blot in 5x SSC. Loosely roll the blot and place inside the hybridization tube.

3. Pour off the 5x SSC and appropriate volume of hybridization buffer.

4. Prehybridize in an oven for 15 minutes-1 hr, at 42°C.

5. Add the labeled probe to the pre-hybridization buffer in the tube. Avoid placing it directly on the membrane and mix gently. Some of the buffer can be withdrawn for mixing with the labeled probe before addition to the buffer. Continue incubation with gentle agitation overnight at 42°C.

6. Prewarm the appropriate volume (2-5 ml/cm^2 of the membrane) of wash buffer to 42°C.

7. Discard the hybridization buffer. Add 50-100 ml of 5x SSC buffer to the tube and replace in the oven for 5 minutes

8. Discard the 5x SSC buffer and replace with primary wash buffer and wash for 20 minutes with gentle agitation at 42°C.

9. Carry out a further wash in fresh primary wash buffer at 42°C for 20 minutes

10. Remove the blot from the hybridization tube, place in a suitable container and cover with an excess of secondary wash buffer. Incubate with gentle agitation for further 5 minutes at room temperature.

11. Discard the secondary wash buffer and replace with an equal volume of fresh secondary wash buffer. Incubate with agitation for further 5 minutes at room temperature.

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XVII. APPENDIX - I

WILDLIFE DISEASES - MATERIAL SAMPLING

S.NO	DISEASE	CLASS OF ANIMALS	LESIONS	MATERIALS TO BE COLLECTED
		BACTERIAL DISEASES		
1.	TUBERCULOSIS	Artiodactylids: Llamas, Deer, Urial, Tapirs and Antelopes, Giraffe, Wild Pigs & Gaur Carnivores: Coati, Binturong, Cat bear, Lion, Tiger, Leopards, Caracals, Slothbear Primates: Monkeys, Baboons, Nilgiri - Langur, Orangutan Proboscida: Elephants Perissodactylids: Rhinoceros Marsupials: Kangaroo Rodents: Porcupine, Flying and Giant Squirrel Reptiles: Python	Nodules of varying sizes in different organs (mostly in lung) and having caseous material or hard and gritty material (Calcification)	Pieces of affected organs in 10% Formal saline along with lymph nodes; impression smear from cut surface of the tuberculous lesions fixed by heat; Tuberculous mastitis: milk from affected quarter in sterile container
2.	JOHNE'S DISEASE	Deers, Gaur	Corrugated appearance of intestinal mucosa (Caecum and Colon)	Heat fixed smears from thickened area of ileocecal valve: small portion of affected bowel in 25% glycerol saline for cultural examination and in 10% formalin along with mesenteric lymph nodes.
3.	NOCARDIOSIS	Hog deer, spotter deer	Lesions akin to tuberculous lesions	Pus smears and histopathology of the lesion reveals the presence of branching granular filaments.
4.	ANTHRAX	Artiodactylids: Gaur, Chital, Hogdeer, Barking deer, Sambar, Wild Pigs, Sikkadeer, Nilgiri Tahr. Carnivores: Leopard, Civet cat, Jaguar. Primates: Lorises Perissodactylids: Rhinoceros Marsupials: Kangaroos.	Discharge of tarry blood from natural orifices. Blood fails to clot. Bloated appearance, splenomegaly	Blood from Jugular Vein in tubes / strips wetted with blood. Transit requires no preservatives. Blood smear from oedematous fluids from the throat / abdomen. Swab of blood / exudate from throat swelling. Muzzle dried in shade for biological test. Putrified case: Piece of ear or muzzle in boric acid

5.	PASTEURELLOSIS	Artiodactylids: Sambars, chital, nilgai, muskdeer, barking deer, blackbuck, chinkara. Carnivores: Red panda, lion, tiger, wolf Primates: Monkeys Perissodactylids: Rhinoceros Reptiles and Amphibians: Giant tortoise	Petechiae on visceral organs, odema of the body and throat swelling (due to odema), lungs – pneumonia, submucosal haemorrhage in the trachea and bronchi	Blood smear or smear from odematous fluid fixed in methanol. Heart blood swab in the sterile tubes containing transport medium or on ice. Small pieces of lung and spleen in sterile container on ice. Long bone if the animals are dead long before. HP: lung, liver, spleen, kidney and heart in 10% formal saline.
6.	ENTEROTOXEMIA	Artiodactylids: Giraffe, deer, guanaco Carnivores: Sloth bear, brown bear Primates	Petechiae or ecchymoses in the epicardium and endocardium, serous surface of the intestine, diaphragm and thymus, hydropericardium, catarrhal gastroenteritis with distension. 'pulpy kidney' focal malacia of brain	Heat fixed impression smear form the affected intestinal mucosa: ingesta from small intestine preserved with 3 or 4 drops of Chloroform: HP: Liver, Lung, Spleen, Kidney, Heart. Stomach and Intestine and other organs showing lesion in 10% Formalin.
7.	BLACK QUARTER	Blackbuck	Affected area - (thigh) skin stiff and black. Foul smelling serosanguineous exudate oozes from the affected quarter. On incision: Muscle is torn, black and contain air bubbles.	Smear from the exudate from thigh. Piece of affected muscle in sterile container on ice and in 10% formalin. Piece of muscle dried in shade.
8.	STRANGLES	Wild ass	Purulent inflammation of upper respiratory tract. Abscesses in sub maxillary and pharyngeal lymph nodes	Smear from the pus and discharges from nasal cavity: pieces of affected lymphnodes, lung, spleen and trachea in 10% formalin.
9.	STREPTOCOCCOSIS	Bison, puma, golden langur, wild ass	Localized abscessed lesions due to trauma.	Pus material from the abscesses
10.	STAPHYLOCOCCOSIS	Tiger, lion, rhinoceros, zebra, elephants	Enteritis and arthritis	Intestinal contents & synovial fluid.
11.	SALMONELLOSIS	Artiodactylids: Sambar Carnivores: Jackal, wild cats, leopard Primates: Monkey, langur, chimpanzee	Gastroenteritis, septicemia	Intestinal contents from ileum and large intestine. Mesenteric lymph node, kidney, gall bladder
12.	SHIGELLOSIS	Carnivores: Tiger Primates: Monkeys	Pyrexia, dysentery, petechial hemorrhages on the epicardium. Severe congestion of intestine with hemorrhagic petechiae	Kidney, lung, liver, placenta and synovial fluid for culture and histopathology.

13.	LEPTOSPIROSIS	Tiger, deer, elephants, lion, sambar, nilgai, blackbuck	Mastitis, nephritis, icterus, hemoglobinuria	Portions of lesions of liver, spleen and kidneys.
14.	PLAGUE	Baboons, monkey, rodents	Dullness, fever and early prostration	Swab and smears
15.	CORYNEBACTERIAL INFECTIONS	Elephant, sangai deer, blackbuck, gharial	Septicemia, suppurative wound	Blood, pus from the wounds.
16.	MYCOPLASMO-SIS	Squirrels	Effusions in pleural cavity. Fibrinous deposits (on solidification of lung)	Lung fixed in formalin, effusion fluids in serum tube, lung and bronchial lymphnode.
	RICKETTSIAL DISEASES			
17.	ANAPLASMOSIS	Himalayan flying squirrels, chital, tiger, barking deer	Splenomegaly, mottled liver, distended gall bladder, enlarged lymphnodes and haemorrhages in heart.	Smears from internal organs - liver, kidney, heart, lungs and brain and blood retained in peripheral vessels.
18.	COXIELLOSIS	Rats, ground shrews, bandicoots, house mouse, water snakes, rat snakes, cobra, python, tortoises, plankton fish, frogs, toads and monitors.	Hepatomegaly, splenomegaly, necrotic foci in liver	Blood smear and swab.
19.	EHRLICHIOSIS	Lion	Few reddish erosions on lower gum and tongue. Patchy congestion, hemorrhages, ecchymoses of non glandular portion of stomach and caecum. Petechiae seen on heart, liver enlarged hard to cut. Lymph node enlarged, odematous and on incision revealed petechiae.	Blood smears.
	FUNGAL DISEASES			
20.	MYCOSES	Artiodactylids: Chital, nilagai, sambar, hog deer, blackbuck Carnivores: Tiger, hyena Proboscidae: Elephants Marsupials: Gray Kangaroo Chiroptera: Bats Reptiles: Gharial	Circular or irregularly shaped scaly to crusty patches of alopecia or broken hairs. Patches may coalesce to form large patches.	Skin scraping from the periphery of the lesions along with some hair roots in tightly stoppered bottle.
21.	AFLATOXICOSES	Bears, hogdeer, nilgai	Severe liver damage, marked bile duct proliferation.	Liver, kidney for toxicology

VIRAL DISEASES				
22.	RABIES	Artiodactylids: Cervids and bovids Carnivores: Tiger, brown, bear, racoon (all warm blooded animals are affected)	No gross lesions and symptoms of excessive salivation, paralysis, dropped jaw, change in behaviour and voice	Impression smears from Hippocampus major of brain fixed in Methanol (10 sec): Brain -One half in 10% Formalin or Zenker solution. Other half in 50% Glycerol Saline.
23.	FOOT & MOUTH DISEASE	Gaur, chitals, bisons, nilgai, antelope, blackbuck, sambar, yaks, mithuns, gazelles, elephant, hog deer, blue bull (cloven footed animals are affected)	Vesicles/ulcers: Buccal mucosa and on the gum, dental pad, tongue, forestomach, inter digital spaces, teat and udder	Vesicles (preferred)and necrosis of tongue and heat in phosphate buffered glycerine.1 g of tissue from a unruptured vesicle in transport medium. (Equal amounts of glycerol and 0.04 M phosphate buffer pH 7.2 - 7.6. HP: affected parts including intestine and heart in 10% Formalin
24.	CANINE DISTEMPER	Panda	Coryza, pneumonia, conjunctivitis, vesicles & pustules on ventral side of the abdomen, hyperkeratosis of digital pad.	Trachea, lung, mediastinal lymphnodes, skin, kidney, brain,and liver in 10% formalin, nasal discharge in 50% glycerolsaline.
25.	AFRICAN HORSE SICKNESS	Wild Ass, Zebra	Hydrothorax, Pneumonia, hydropericardium, myocardialnecrosis, Gastroenteritis	Pleural and pericardial fluid, heart blood, affected lung, kidney, spleen, lymphaglands in 50% Glycerin saline oron ice and in 10% Formalin
26.	INFECTIOUS PERITONITIS	Tiger	Large amount of viscous straw or orange colored exudate with abundant fibrin in the abdominal and thoracic cavities	Swabs / lesions
27.	INFECTIOUS HEPATITIS	Panther, leopard, cheetah, lion	Yellow discoloration of body fluids and tissues particularly liver and fat depots. Urinary bladder distended with dark yellow colored urine.	Liver for histopathology in 10% formal saline and 50% glycerol saline
28.	KYASANUR FOREST DISEASE	Monkeys	Hemorrhages in lung, kidney, brain, adrenals. Blood clots and swelling at the anal region were usually present	Liver, lymphnodes, kidneys and brain in 10% formal saline and 50% glycerol saline
29.	POX	Chimpanzee, Elephants	Pox lesions in (vesicles, pustules, scabs)udder and teat -(cow), face, underneath the tail, udder, teat, caseous exudate in lungs (sheep)	Scab in 50% Glycerine and in 10% Formalin.

PROTOZOAN DISEASES				
30.	BABESIOSIS	Artiodactylids: Deer, American bison, Mithun Carnivores: Jackal, wildcat, leopard, white tiger Primates: Monkeys Proboscidae: Elephants Rodents: Indian gerbil	Icterus, low viscosity of blood, enlargement of liver and spleen, gastroenteritis, oedematous lung, urinary bladder contains red or coffee colored urine	Peripheral blood smear (thick) impression smears (thin) from heart blood, brain, kidney, liver,spleen, lungs and blood retained in blood vessels fixed in methanol. Affected ortans in 10% formalin.
31.	THEILERIOSIS	Hedge hogs, chital, Gaur	Enlargement of lymphnode. Pulmonary odema emphysema, petechiae and ulcers in the mucosa of abomasum. Koch's blue bodies in the lymphocytes.	Smears from lymphnodes and peripheral blood smear. Abomasal ulcer, impression smear fixed in methanol, internal organs and Mesenteric lymphnodes in 10% formalin.
32.	TOXOPLASMOSIS	Himalayan giant squirrels, rhesus monkeys, civet cat.		Brain in 10% formal saline
33.	TRYPANOSOMIASIS	Tiger, Lion, Wild dogs, Wolf, Jackals, Deer, Bison, Camel, Elephant, Mangoose etc.	Anemia, enlargement of lymph nodes, Splenomegaly, Inflammatory exudate in pericardium	Peripheral blood smears, impression smears from spleen, and lymph nodes. Affected organs in 10% formalin
34.	SARCOSPORIDIOSIS	Artiodactylids: Goral, Sambar deer Primates: Rhesus macaque Carnivores: Lion jackal, leopard Perissodactyla: Rhinoceros	Sarcocystis in heart muscle	Cyst containing tissue in 10% formalin
35.	COCCIDIOSIS	Rare in wild animals	Catarrhal / haemorrhagic enteritis	Intestinal mucosal scrapping and intestinal tissues
36.	ENTAMOEBIASIS	Lion, monkey, chimpanzee, yellow baboon, Indian turtle.	Enteritis	Fecal samples
37.	BALANTIDIASIS	Monkeys, Orangutan	Enteritis	Fecal samples
38.	GIARDIASIS	Lion	Enteritis	Fecal samples
39.	LEISHMANIASIS	Gerbils	Enteritis	Fecal samples

APPENDIX - II

PROFORMA FOR POST-MORTEM EXAMINATION

NOTES ON POST-MORTEM EXAMINATION ON THE BODY OF A .....
BELONGS TO .....
.....

Result of examination of blood smear taken after death --

A. General Information

1. Date of admission
2. Date and time of death
3. Date and time of making PM Examination
4. History
5. Clinical diagnosis
6. Environmental & Surrounding Evidences

B. External Examination

1. Class of animal, sex, age, species
2. Descriptive marks
3. Condition of the body
4. Rigor mortis
5. Natural orifices
6. Visible mucous-membranes
7. Presence of wounds, if any
8. Superficial lymph glands
9. Any other abnormalities

C. Internal Examination

I. Subcutaneous tissue

II. Abdominal Cavity

1. Peritoneal cavity and peritoneum
2. Position of organs (organs to be merely inspected and none removed at this stage.
3. Any other abnormality

III. Thoracic Cavity

1. Pleural cavity and pleurae
2. Position of organs
3. Any other abnormality

IV. Pericardial sac

V. Heart

1. Gross appearance, Colour, Size, etc.
2. Chambers
3. Valves
4. Myocardium
5. Blood vessels

VI. Lungs

1. Gross appearance, Colour, Size, etc.
2. Palpable abnormalities
3. Section
4. Parasites
5. Lymph glands

VII. Trachea and bronchi

1. Abnormalities
2. Parasites
3. Bronchial lymph glands

VIII. Larynx

1. Abnormalities
2. Parasites

IX. Diaphragm

X. Liver

1. Gross appearance colour, size, etc.
2. Surface
3. Borders
4. Parenchyma
5. Lymph glands
6. Gall bladder - (Wall and contents)
7. Parasites

XI. Spleen

1. Gross appearance, colour, size, etc.
2. Surface
3. Section
4. Parasites

XII. Kidneys

1. Gross appearance, colour, size, etc.
2. Capsule
3. Renal surface
4. Section
5. Renal pelvis
6. Parasites and calculi

XIII. Adrenal glands

XIV. Mouth (lips, teeth, gums, palate etc.)

XV. Tongue

XVI. Pharynx

1. Submaxillary L.glands
2. Associated glands tonsils, retro pharyngeal, supra pharyngeal L.glands

XVII. Oesophagus

1. Parasites

XVIII. Stomach (s)

1. Outer surface
2. Interior and contents
3. Parasites

XXIX. Intestines

1. Mesentery, mesenteric blood vessels and parasites
2. Wall and its surfaces (serous and mucous)
3. Ileocecal valve
4. Contents
5. Parasites
6. Lymph glands

XX. Pelvic cavity

1. Urinary bladder
2. Generative organs
3. Lymphatic glands

XXI. Brain and spinal cord

XXII. Skeleton

XXIII. Musculature

XXIV. Clinical laboratory examinations

1. Blood smear
2. Brain smear
3. Other examination
4. Appearances found

Etiological diagnosis

Remarks

Place

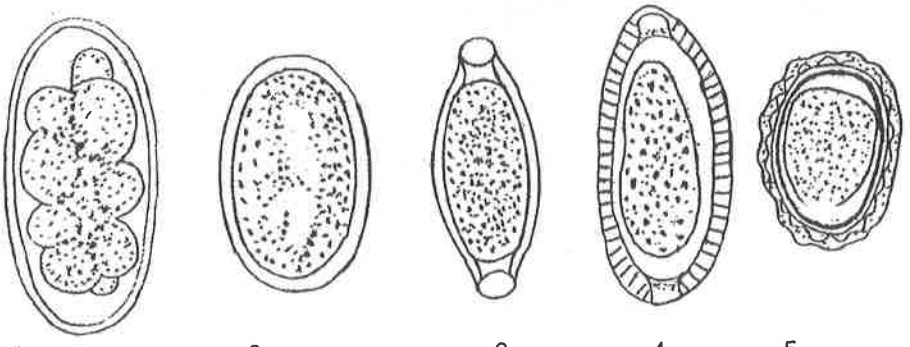
Date

Signature

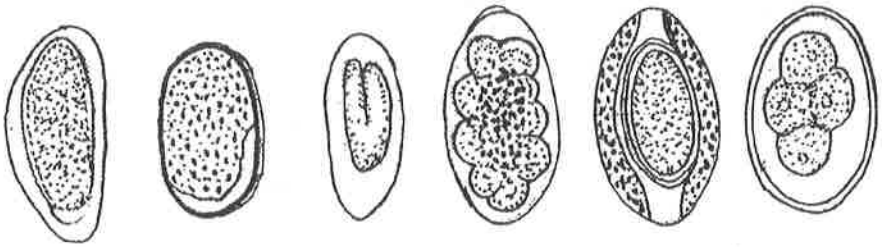
Designation of Veterinary Officer

APPENDIX-III - PARASITIC OVA OF WILD ANIMALS

PARASITE OVA OF PRIMATES



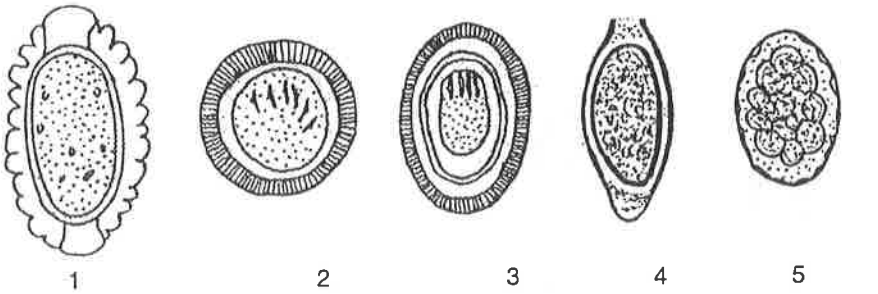
1 2 3 4 5



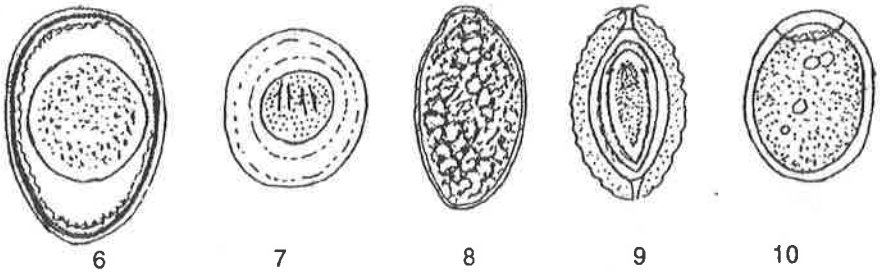
6 7 8 9 10 11

1. Oesophagostomum sp.
   60 - 63 by 27 - 40 µm
2. Physaloptera sp.
   42 - 60 by 29 - 42 µm
3. Trichuris sp.
   50 by 22 µm
4. Capillaria sp.
   48 - 62 by 29 - 37 µm
5. Ascaris sp.
   45 - 75 by 35 - 50 µm
6. Streptopharagus sp.
   28 - 38 by 17 -22 µm
7. Molineus sp.
   40 -52 by 20 -29 µm
8. Enterobius sp.
   50 -60 by 20 -32 µm
9. Trichostrongylus sp.
   75 - 86 by 34 - 45 µm
10. Prosthenorchis sp.
    42 - 53 by 65 - 81 µm
11. Ancylostoma sp.
    60 by 40 µm

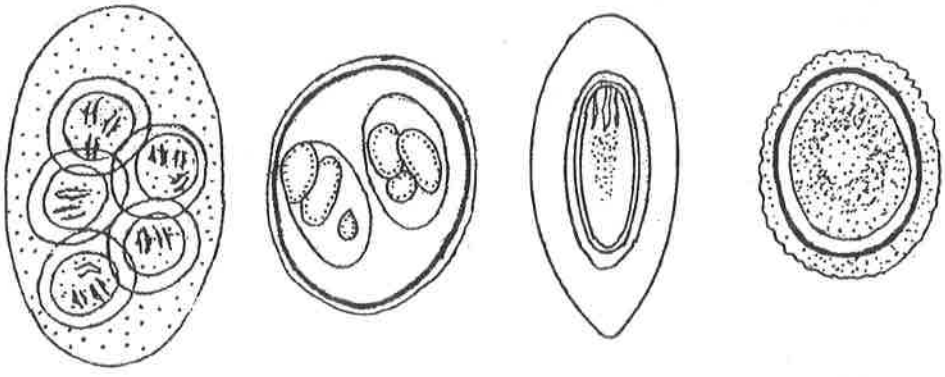
PARASITE OVA OF CARNIVORES



1 2 3 4 5



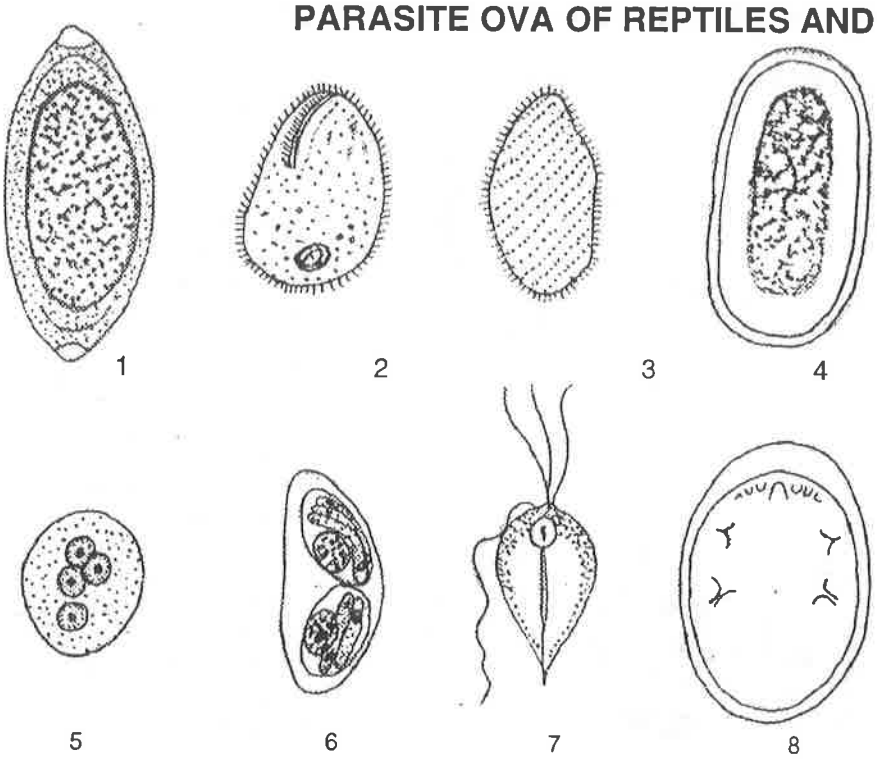
6 7 8 9 10



11 12 13 14

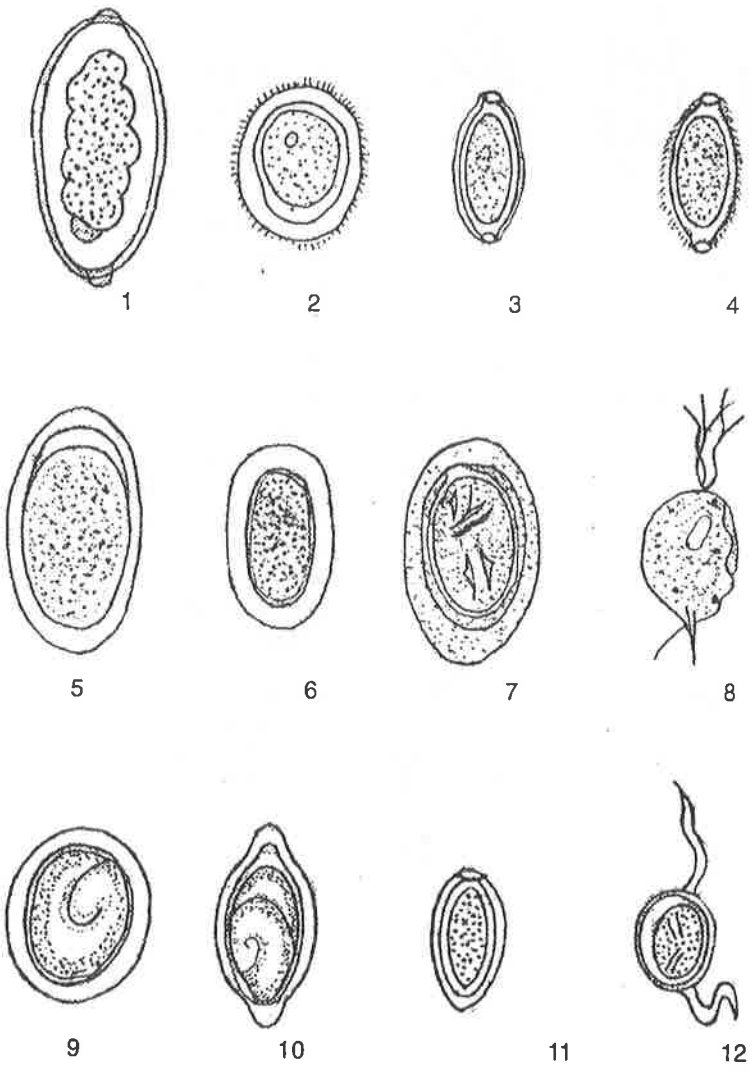
1. Dioctophyma sp.
   71 - 84 by 45 - 52 µm
2. Taenia and Echinococcus sp.
   30 - 50 by 20 - 37 µm
3. Oncicola sp.
   60 - 70 by 40 -50 µm
4. Trichuris sp.
   70 - 89 by 37 - 40 µm
5. Ancylostoma sp.
   55 - 75 by 34 - 47 µm
6. Toxascaris sp.
   75 - 85 by 60 -75 µm
7. Hymenolepis sp.
   44 - 62 by 30 - 53 µm
8. Paragonimus sp.
   75 - 118 by 42 - 67 µm
9. Acanthocephala sp.
10. Diphyllobothrium sp.
    67 - 71 by 44 - 45 µm
11. Dipylidium sp.
    58 by 45 to 260 by 170 µm
12. Isospora sp.
    39 - 44 by 29 - 35 um
13. Moniliformis sp.
    85 - 118 µm (length)
14. Toxocara sp.
    90 by 75 µm

PARASITE OVA OF REPTILES AND AMPHIBIANS



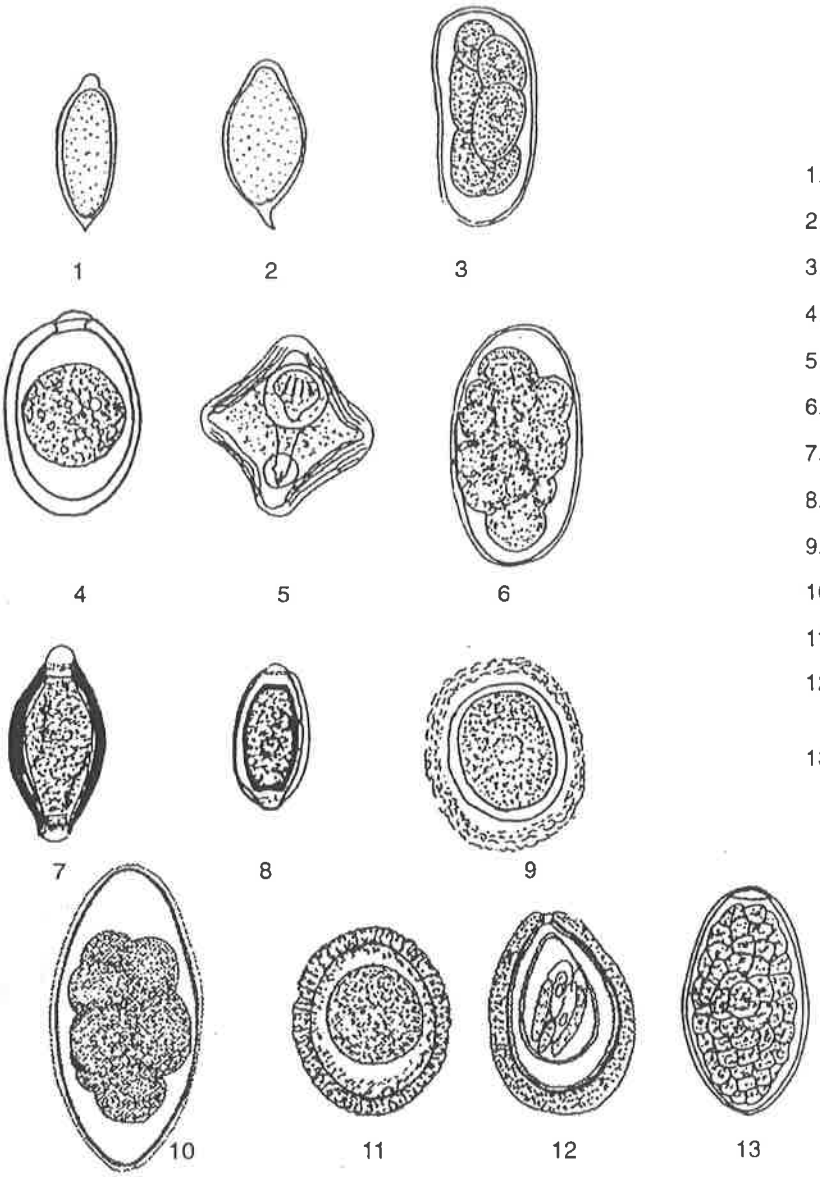
1. Capillaria sp.
   48 - 65 by 23 - 28 μm
2. Balantidium duodeni
   70-80 μm
3. Opalina sp.
   400 - 840 by 175 - 180 μm
4. Kalicephalus sp.
   80 - 100 by 40 -60 μm
5. Entamoeba cyst
   11- 20 μm (diameter)
6. Isospora sp.
   50 by 25 μm
7. Monocercomonas sp.
   15 by 6 μm
8. Pentastomid sp.
   70 - 90 μm

PARASITE OVA OF AVES



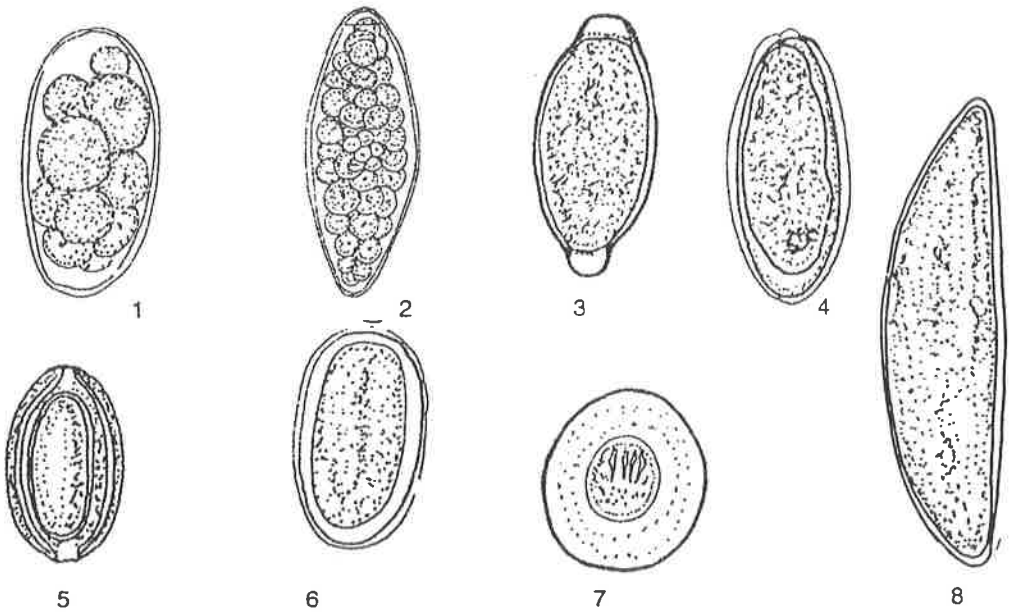
1. Syngamus sp.
   70 - 100 by 43 - 46 μm
2. Contracaecum sp.
   60 - 75 by 50 - 60 μm
3. Capillaria sp.
   48 - 65 by 23 - 28 μm
4. Capillaria sp.
   48 - 65 by 23 - 28 μm
5. Ascaridia galli
   73 - 92 by 45 - 57 μm
6. Heterakis gallinae
   65 - 80 by 35 - 46 μm
7. Metroliasthes sp. 25 μm
8. Trichomonas gallinarum
   9 -15 by 5 -9 μm
9. Subulura sp.
   52 - 64 by 41 - 49 μm
10. Tetrameres sp.
    35 - 40 by 23 - 29 μm
11. Prothogonimus sp.
    25 - 32 by 10 - 15 μm
12. Choanotaenia sp. 400 μm

PARASITE OVA OF RUMINANT AND EQUINE



1. Ornithobilharzia sp. 72 - 77 by 18 - 26 μm
2. Schistosoma sp. 132 - 247 by 38 -60 μm
3. Bunostomum sp. 79 - 106 by 46 - 50 μm
4. Eimeria sp. 80 - 88 by 55 - 59 μm
5. Moniezia sp. 56 - 57 μm (diameter)
6. Strongyle sp. 70 - 85 by 40 - 47 μm
7. Trichuris sp. 70 - 80 by 30 - 42 μm
8. Capillaria sp. 50 by 25 μm
9. Ascaris sp. 50 - 75 by 40 - 50 μm
10. Nematodirus sp. 175 - 260 by 106 110 μm
11. Parascaris sp. 90 - 100 μm
12. Eimeria leuckarti (equine)
    80 - 87 by 55 - 59 μm
13. Gastrothylax sp. 115 - 135 by 66 - 70 μm.

PARASITE OVA OF LABORATORY ANIMALS



1. Oesophagostomum sp.
   69 - 86 by 35 - 55 μm
2. Gastrodiscoides sp.
   150 - 152 by 60 - 72 μm
3. Trichosomoides sp.
   60 - 67 by 40 - 48 μm
4. Passalurus sp.
   95 - 103 by 43 μm
5. Prosthenorchis sp.
   42 - 53 by 65 - 81 μm
6. Abbreviata sp.
   39 - 50 by 23 - 34 μm
7. Hymenolepis sp.
   44 - 62 by 30 - 53 μm
8. Syphacia sp.
   118 - 153 by 33 - 55 μm