LABORATORY MANUAL

VETERINARY PATHOLOGY

VPP- Unit-4, 5 & 6

COURSE TITLE – PATHOLOGY OF INFECTIOUS AND NON-INFECTIOUS DISEASES OF DOMESTIC ANIMALS, AVIAN PATHOLOGY, PATHOLOGY OF DISEASES OF LABORATORY AND WILD ANIMALS

Compiled By:

Prof. (Dr)S. D. Singh
(Incharge&HOD)

Prof. (Dr)Nem Singh
(Professor)

Prof. (Dr) G. D. Sharma
(Professor)

Dr. Aditi Kumawat
(Assistant Professor)

Name – ……..

Roll No / Enroll No -……/……

Batch -……..

Session –……..

Department of Veterinary Pathology
Mahatma JyotibaFule College of Veterinary & Animal Sciences Chomu, Jaipur

FOREWORD

I am glad to see the Laboratory Manual GENERAL PATHOLOGY, SYSTEMIC PATHOLOGY, ANIMAL ONCOLOGY, CLINICAL PATHOLOGY AND NECROPSY is prepared by Dr. S. D. SinghProf. & Head, Dr. Nem SinghProfessor, Dr.Prof. (Dr) G. D. Sharma Professor, and Dr. Aditi Kumawat Assistant Professor, Dept. of Veterinary Pathology. It is appreciable to note that the manual covers the practical syllabus of B.V.Sc.& A.H. course as per the standards laid down by Veterinary Council of India.

Dr S. D. Singh, Dr.Nem Singh, Dr.) G. D. Sharmaand Dr. Aditi Kumawat have devoted keenly to prepare this manual with their excellent knowledge and expertise in the field of pathology. They have covered all the aspects like objectives, outline and description, material and methods and observation to be taken care off.

Definitely this manual will be helpful for smooth and effective conduction of practicals and ensure a handbook for students for entire life in the profession.

I congratulate Dr.S. D. Singh, DrNem Singh, Dr.Ajay Kumar Thakan and Dr. Aditi Kumawat for their strenuous efforts and excellent presentation of this manual.

Dean

Mahatma JyotibaFule College of
Veterinary & Animal Science, Chomu, Jaipur

PREFACE

This Manual has been prepared for the undergraduate students of B.V.Sc. & A.H. in accordance with the syllabus designed by the Veterinary Council of India. The efforts have been made to make the manuscript worthy, realistic and easily understandable for the students, teachers and Veterinary Practitioners for diagnosis of different pathogens of diseases of animals. We hope this manual will serve very useful tool to the undergraduate and graduate students of Veterinary Science who are undergoing courses in veterinary Pathology.

It's our pleasure to thank Dean Sir, M.J.F College of veterinary and Animal Sciences, Chomu, Jaipur for providing necessary facilities and rendering all helps in preparing this course manual.

Computer operator and typist Mr. Mukesh Kumar Saini and Mr. Ashutosh Sharma worked hard for very existence of this manual so I acknowledge his efforts.

Course Incharge

Dept. of Veterinary pathology

UNIT -4 PATHOLOGY OF INFECTIOUS AND NON-INFECTIOUS DISEASES OF DOMESTIC ANIMALS

S. No.	Date	Exercise	Signature of Teacher

UNIT- 5 AVIAN PATHOLOGY

S.N.	Exercise	Date	Remarks

S.N.	Exercise	Date	Remarks

EXERCISE NO. 1. DIAGNOSTIC CYTOLOGY

Diagnostic cytology is a very useful tool for diagnosis of various disease conditions especiallyneoplastic diseases. The cytological interpretation is very important in establishing adiagnosis,identifying the disease process, directing the therapy and predicting the prognosis. The technique is almost non-invasive. Cytologic evaluation can also correlated with other clinical and laboratory findings to arrive at a definitive diagnosis.

Collection of Cytological Samples:

The following cytological techniques are used depending upon the disease conditions.

Fine needle aspiration:

It is performed by using a standard syringe (5,10ml) & needle(22, 23gauge) depending on the size & consistency of the given skin and proliferative masses. The needle is inserted into the masses after proper disinfection of the presented area with alcohol and then withdrawn quickly. The collected material then transferred to the one end of properly cleaned glass slide. Different types of smear preparation technique like blood smear technique, squash preparation & starfish preparation are followed depending on the nature of the aspirated material. The smears are air dried quickly at room temperature during summer & artificial heat is used in winter season.

Impression smears or tissue imprints:

Firstly the presented site is properly cleaned & disinfected by using alcohol and the site is blotted with clean tissue paper until no blood oozed out. Then several tissue imprints or impression smears are prepared on clean microscopic glass slides from exudative or ulcerated mass. Sometimes,tissue imprints are also taken from the cut surface of incisional and excisional biopsies at postmortem.

Scrapings

This technique is employed when nothing is aspirated from dry & hard tissues by standard needles and impression smears failed. Skin scrapings are collected repeatedly from exposed sites ofpatient having alopecia, itching & erythematous lesions, till blood oozes out in a vial containing 10%of potassium hydroxide (KOH). This technique is also applied on some external lesion or tissues obtained after surgery.The materials taken on

the blade or edge of the glass slide used for scraping is then transferred to the middle of a clean glass slide & spread to get monolayer of cells.

Direct microscopic examination for fungi and mites:

Little amount of collected skin scrapings are placed on a clean glass slide. Then few drops of10% KOH are poured on that slide and covered it by a clean cover slip. To clear the debris, the slideis now gently heated for 15-20 minutes. Ultimately, the prepared slide is observed under microscopein both low &high power magnification to detect the presence of fungal elements. Again, the slide isexamined at lower magnification (10x) for presence of mites. In this way, identification of *Demodex canis* and *Sarcoptes scabiei* can be done easily as per standard methods.

Harris Hematoxylin

1000mL

Hematoxylin, Potassium Alum, Glacial Acetic Acid, Sodium Iodate. Filter before use.EA-50(EA-65) OG-6 were developed as generalstains.

StorageandStability:

Storeat15°-30°C

WarningsandPrecautions:

Papanicolaou Staining reagents are flammable and toxic. Keep away from sources ofignition. In case of contact with eyes, rinse immediately with water and seek medical advice.Maybe fatal or cause blindness if swallowed.

Dispose of waste in accordance with applicable,laws.

Materials,Required:

Ethanol, HydrochloricAcid, AmmoniumHydoxide, Xylene,Methanol, Coverslips, Microscopeslides and microscope.

Specimen Collection:

In the collection and preparation of smears for cytological examination, the major objectives are:

1. Specimens should have as ufficient number of cells from the area in question.
2. Smears should contain well preserved cells uniformly distributed so that eachcell can be individually examined.
3. The staining procedure should clearly define the details of all structures.

Cytological preparations are obtained from patient by approved methods and techniques. Scraping, obtained from the vagina, uterus, cervix, mouth or ulcerated skin area is spreaddirectlyon a clean microscope slide.

Sediment (obtained by centrifugation or filtration) from bodily secretions is spread on a

clean microscope slide. The smear is immediately fixed with a cytological spray fixative or in analcohol-ether dip. Fixation or preservation is one of the most important steps in the procedure.Drying of the cells prior to fixation will usually result in artifacts such as nuclear distortion and vacuolization. After fixation there are no special handling requirements for cytological smears.However, smears which are to be mailed to a laboratory should remain in the fixative for about onehour.Asecond cleanglassslide maybe placed oneach fixed slidefor protection.

Procedure:

Notes:

Filter the Harris Hematoxylin immediately before use.

1. Dip slide(s) gently 5-10times in 95% ethanol.
2. Dip slide(s) gently5-10times in 70%ethanol.
3. Dip slide(s)gently5-10times in distilled water.
4. Stain 5 minutes in Harris Hematoxylin.
5. Place smears in distilled water. Rinse in successive changes of distilled water until the water remains colourless.
6. Dip slide(s) gently 5-10times in 70% ethanol.
7. Dip slide(s) in a 1% solution of HCl in 70% ethanol until the smear shows as al mond colour.
8. Rinse slide(s) well in 2changes of 70%ethanol.
9. Dip slide(s) gently in a 3% solution of ammonium hydroxide in 70% ethanol until the smear takes on a blue colour.
10. Rinse the slide(s)in two changes of 70%ethanol.
11. Dip slide(s)5-10timesi n 95%ethanol.
12. Stain slide(s)in OG-6for 2minutes.
13. Rinseslide(s) in two changes of 95%ethanol.
14. Stainslide(s) in EA-50 or EA-65 for 3 -6 minutes.
15. Rinse slide(s) well in two changes of 100% methanol.
16. Rinse slide(s)in one part absolute methanol one part xylene.
17. Clean smear in xylene.

Mounting Procedure:

1. After the smear has been completely cleaned in xylene it is mounted with a

microscope slide coverglass preferably22x40mm, #1 thinness.

2. A permanent clean mounting medium should be used.

3. The excess xylene should be drained, in order to avoid the appearance of airspaces when xylene evaporates.

4. Place the required amount of mounting medium along an edge of one of the longer borders of the coverslip.

5. Place the slide at right angles to the edge of the coverslip so that the side containing the cells is facing the mounting medium.
6. Slowly lower the slide and permit the mounting medium to spread between the slide and coverslip.

Results:

Nuclei are stained blue while cytoplasm displays varying shades of pink, orange, yellow and green.

Limitations:

1. Proper specimen collection and fixation of cells is essential for interpretation.

Examination of Slides:

1. Examine the smears at low power,evaluate the area having good number of intact properly stained cells.
2. Identify the type of cell population. In case of inflammatory reaction,the possible etiologic agent may be as certained.
3. Presence of large noninflammatory cells in the cytological specimens suggest proliferative tissue mass.
4. Any evidence of malignancy should be determined by the type of cells present.

EXERCISE NO. – 2 **FrozenSectioning**

The frozen section procedure is a pathological laboratory procedure to perform rapid microscopic analysis of a specimen. It is used most often in oncological surgery. The technical name for this procedure is cryosection.

Then intra operative on siltation I s the name given to the whole intervention by the pathologist, which includes not only frozen section but also gross evaluation of the specimen, examination of cytology preparations taken on the specimen (e.g. touch imprints),and aliquoting of the specimen for special studies(e.g.molecular pathology techniques,flow cytometry). The report given by the pathologist is usually limited to a "benign" or"malignant"diagnosis, Traditionally shouted into an intercom.

Cryostat

History

The frozen section procedure as practiced today in medical laboratories is based on the description by Dr Louis B.Wilson in 1905.Wilson developed the technique from earlier reports at the request of Dr William Mayo, surgeon and one of the founders of the

Mayo Clinic. Earlier reports by Dr Thomas Cullen in at Johns Hopkins Hospital in Baltimore also involved frozen section, but onlyafter formalin fixation, and pathologist Dr William Welch, also at Hopkins,experimented with Cullen's procedure but without clinical consequences. Hence, Wilson is generally credited with truly pioneering the procedure.

Uses of frozen sectioning

- The principaluseof the frozen section procedure is the examination of tissue while surgery is taking place.This may befor various reasons:
- If a tumor appears to have metastasized, a sample of the suspectedmetastasis is sent for cryosection to confirm its identity. This will help the surgeon decide any point in continuing the operation. Usually, aggressive surgery is performed only if there is a chance to cure the patient. If the tumor has metastasized, surgery isusually not curative, and the surgeon will choose a more conservative surgery,or no resection at all.
- If a tumor has been resected but theneedtomake a further resection for clear margins it is unclear whether the surgical margin is free of tumor, an intraoperative consultation is requested to assess.

- In a sentinel node procedure, a sentinel node containing tumor tissue prompts a further lymph node dissection, while a benign node wil lavoid sucha procedure.
- If surgery is explorative, rapid examination of a lesion might help identify the possible cause of a patient's symptoms. It is important tonote, however, that thepathologist Is very limited by the poor technical quality of the frozen sections. A final diagnosis is rarelyofferedintraoperatively.
- Rarely, cryosections are used to detect the presence of substances lostin the traditional histology technique, for example lipids. They canalsobe used to detect some antigens masked by formalin.

Preparation of Frozen Sections for Sectioning

Materials needed:

2-Methyl butane (isopentane) Liquid Nitrogen

Dryice

Peel-Away base molds

Frozen tissue matrix (OCT or Cryo matrix) Long forceps

Necropsy tools

slides

Procedure-1st:

- The key instrument for cryosection is the **cryostat**, which is essentially a

microtome inside a freezer.

- The surgical specimen is placed on a metal chuck and frozenrapidly to about-20°C.
- At this temperature, most tissues become rock-hard.
- Subsequently it is cut frozen with the microtome portion of the cryostat, the section is picked up on a glass slide and stained (usually with hematoxylin and eosin, the H&E stain).

- The preparation of the sample is much more rapid than with traditional histology technique (around 10 minutes vs 16hours).
- However, t he technical quality of the sections is much lower.

Sectioning of Frozen Tissues

- Before cutting sections, allow the temperature of the block to equilibrate to the temperature of the cryostat (typically-20Â°C).
- Place the tissue block on the cryostat specimen disk.
- Adjust th\e positioning of the block to align the block with the knife blade.
- Cut tissue block until the desired tissue is exposed.
- Cut sections of the desired thickness (usually5Âµm), place the sections on a Fisher Superfrost slide and dry over night at room temperature(RT).
- Fix slides by immersion in cold acetone (-20Â°C) for 2 minutesor other suitable fixative (e.g. alcohol, formal alcohol, formalin,etc.),air dryat RT and proceed tostaining.

- Alternatively, the frozen section slides can be stored for a short periodof time at -70Â°C in a sealed slide box. When ready to stain, removeslides from freezer and warm to-20Â°C in the cryostat or-20Â°freezer, fix for 2 minutes in cold fixative (acetone or other suitable fixative) and allow to come to RT to continue with the staining.

Biopsy–Types and Methods

Biopsy is the technique of collection of tissue specimens from an ailing animal. Biopsy helps to diagnose and determine diseases and initiate the treatment. There are varioustechnique and equipments for biopsy procedures. These include aspiration by needle,excisional or incisional biopsy, and exfoliative cytology, including irrigation of holloworganand serous cavities,direct smears, scrapingsand curettage.

1. **Exfoliative Cytology –** In this method, cells are collected after they have been either spontaneously shed by the body ("spontaneous exfoliation") or manually scraped/brushed off of a surface in the body *i.e.* "mechanical exfoliation".

   An example of spontaneous exfoliation is when cells of the pleural cavity or peritoneal cavity are shed into the pleural or peritoneal fluid. This fluid can be collected *via* various methods for examination.

   Examples of mechanical exfoliation include Pap smears, where cells are scraped from the cervix with a cervical spatula, or bronchial brushings, where a bronchoscope is inserted into the trachea and used to evaluate a visible lesion by brushing cells from its surface and subjecting them to cytopathologic analysis.

2. **Intervention cytology-** In interventional cytology the pathologist intervenes into the body for sample collection. Presently, FNAC has become synonymous to interventional cytology.

1. **Fine-Needle Aspiration Cytology orNeedle aspiration biopsy–** A needle attached to a syringe is used to collect cells from lesions or masses in various body organs by microcoring, often with the application of negative pressure (suction) to increase yield. FNAC can be performed under palpation guidance (i.e., the clinician can feel the lesion) on a mass in superficial regions like the neck, thyroid or breast; FNAC may also be assisted by ultrasound or CAT scan for sampling of deep-seated lesions within the body that cannot be localized via palpation. FNAC is widely used in many countries, but success rate is dependent on the skill of the practitioner.

Fine needles are 23 to 27 gauge. Because needles as small as 27 gauge can almost always yield diagnostic material, FNAC is often the least injurious way to obtain diagnostic tissue from a lesion. Sometime a syringe holder may be used to facilitate using one hand to perform the biopsy while the other hand is immobilizing the mass. Imaging equipment such as a CT scanner or ultrasound may be used to assist in locating the region to be biopsied.

2. Sediment cytology– In this, the sample is collected from the fixative that was used for processing the biopsy or autopsy specimen. The fixative is mixed properly and taken into a centrifuge tube and is centrifuged. The sediment is used for smearing. These sediments are the cells that are shed by the autopsy and biopsy specimen during processing.

Parameters

The nucleus of the cell is very important in evaluating the cellular sample. In cancerous cells, altered DNA activity can be seen as a physical change in the nuclear qualities. Since more DNA is unfolded and being expressed, the nucleus will be darker and less uniform, larger than in normal cells, and often show a bright-red nucleolus. Therefore, there is presence of hyperchromatic nuclei in the cases of malignancies.

While the cytologist's primary responsibility is to discern whether cancerous or precancerous pathology is present in the cellular sample analysed, other pathologies may be seen such as:

- microbial infections: parasitic, viral, and/or bacterial
- reactive changes
- immune reactions
- cell aging
- amyloidosis
- autoimmune diseases

Various normal functions of cell growth, metabolism, and division can fail or work in abnormal ways and lead to diseases.

Cytopathology is best used as one of three tools, the second and third being the physical exam and imaging. Cytology can be used to diagnose a condition and spare a patient from surgery to obtain a larger specimen. An example is thyroid FNA; many benign conditions

can be diagnosed with a superficial biopsy and the patient can go back to normal activities right away. If a malignant condition is diagnosed, the patient may be able to start radiation/chemotherapy, or may need to have surgery to remove the cancer.

Some tumors may be difficult to biopsy, such as sarcomas. Other rare tumors may be dangerous to biopsy, such as pheochromocytoma. In general, a fine-needle aspiration can be done anywhere it is safe to put a needle, including liver, lung, kidney, and superficial masses.

Sometimes more information about the specimen is helpful. Immuno-histochemical stains and molecular testing can be performed, especially if the sample is prepared using liquid based cytology.

Collection of samples in various common diseases of animals

Bacterial Diseases

S. No.	Name of disease	Material to be collected
1.	Tuberculosis	i) Small piece of different organs showing lesions preserve in10 percent formalin. ii) Impression smears from cut surface of tuberculous lesions fixed by heat. iii) In tuberculous mastitis milk from affected quarter in a sterile vial on ice.
2.	Johne'sDisease(J. D.)	i) Smears of mucosa & tissues pinched from rectum and pressed over the slide with the help of other slide and fixed by gentile heat. ii) Faecal sample removed from the rectum in a sterile vial kept on ice. iii) From dead animal smears from scrapings of ileocaecal junction, impression smears of affected part of intestine and rectal mucosa, lymph nodes and liver fixed by gentle heat. iv) Small pieces of affected part of intestine from ileocaecal mesenteric lymph nodes in 10% formalin.
3.	Actionomycosis	i) Smears of pus from deeper portions of lesions fixed by heat. The smear prepared in a similar manner as in the case of actinobacillosis. ii) Pus in a sterile vial. iii) From dead animal, small pieces of affected organ/tissue preserved in 10 % formalin taken for histopathological examination.
4.	Actinobacillosis	i) Smears of pus from deeper portions of the lesions prepared

		by crushing the pus granules. Smears fixed by gentle heat before dispatch. ii) Pus in a sterile vial. iii) From dead animal, small pieces of affected organ/ tissues, preserved in 10% formalin for histopathological examination.
5.	Anthrax	i) Blood smears from the tp of the ear vein or tail, fixed by gentle heat. ii) Swab of blood from the ear vein or exudates from swelling in a sterile test tube, sealed immediately. iii) Small piece of ear or muzzle in boric acid or in a suitable tight-fitting container for Ascoli's test. Particularly in Purified carcasses.
6.	Black Quarter (BQ)	i) Smears prepared from fluid collected from the swelling of the affected muscles region and fixed by heart. ii) From dead animal a small portion of affected muscles in a sterile sample collection bottle. Sent on ice through a messenger. iii) Impression smears from the affected muscles, dried and fixed by heart. iv) Two inch cube of affected muscles and other organs showing lesions to be collected in 10 % formalin.
7.	Haemorrhagic septicaemia (H.S.)	i) Blood smears, fixed by methanol for 3 minutes. ii) Smears from oedematous fluid, fixed by methanol for 3 minutes. iii) blood swab from heart for culture in a sterile test tube containing transport medium or on ice. iv) About 10 ml blood in a sterile vial for animal inoculation , transported on ice with a messenger.

		v) A small piece of lung and spleen 2” to 3” square, in a sterile container on ice. vi) Long bone packed in charcoal enclosed in plastic bag. vii) For hsitopathological studies small pieces from lung heart, liver, spleen, kidney, lymph node preserved in 10 % formalin.
8.	Leptospirosis	i) Freshly voided urine in test tube on ice. ii) Blood collected during febrile stage of disease in test tube on ice sent through messenger. iii) Serum sample in a test tube on ice. iv) From autopsy pieces of kidney, liver, spleen and organs showing lesions, in 10 % formalin.
9.	Listeriosis (associated with encephalitis and septicemia)	i) Portion of brain, lung, liver, spleen and kidney in a tube on ice sent through messenger. ii) Small pieces of above organs in 10 % formalin.
10.	Abortion(Brucella/ Leptospira/vibrio/ Chlamydia)	a) Placenta i) Impression smears prepared from at least 2-3cotyledon after drying the cotyledons s with filter paper. These are to be air-dried and gently heat-fixed (2 slides) and methanol fixed (2 slides) ii) Small pieces fro 2-3 cotyledons in a sterile bottle on ice. Sent through a messenger. iii) Small piece from thoracic and abdominal and interocotyledone membranes, preserved in 10% formal saline. b) Foetus iv) Smears from thoracic and abdominal fluid air-dried and gently heat-fixed. v) Small pieces from lung liver, spleen, kidney and brain in a

		sterile bottle on ice, sent through a messenger. vi) Thoracic fluid (5ml) on ice for cultural isolation. Vii) Foetal stomach after tying its both ends and kept in a sterile bottle on ice or 10-20 ml of stomach content in sterile vials on ice sent through a messenger for isolation of bacteria. viii) Small pieces of lung, heart, liver, spleen, thymus. Stomach, kidney, brain and intestine from foetus preserved in 10 % formal saline. c) Mother ix) Vaginal smears, prepared within 3 days of abortion air-dried and gently heat- fixed. Smears prepared from deeper part of vagina and fixed heat. x) Serum samples collected asceptically on the day of abortion and 3 weeks after the abortion. About 10 ml serum needed (for which about 40 ml of blood collected) Clean serum sent in a sterile vial once, needed for serological testes. xi) For *Brucella abortus* ring test, milk should be collected avoiding colostrums and from drying off animals or from those suffering from mastitis. xii) Uterine discharge collected in sterile tubes from the aborted animals and kept on ice. d) Sire xiii) Preputial washing in a sterile container kept on ice for vibriosis.
11.	Mastitis	i) Midstream milk samples collected from each test in separate sterilized vials after test orifice cleaned with alcohol, sent on ice after labelling RH. LH. RF. LF.
12.	Vulvitis, Vaginitis, Metertis	i) Vaginal swab in transport medium and vaginal discharge in sterile vials on ice for cultural examination and drug

		sensitivity tests.

Viral Diseases

S. No.	Name of disease	Material to be collected
1.	Food and Mouth Disease	i) Necrosed part of the tongue preferably epithelium and /or foot lesions in 3 to 5 gms quantity collected in phosphate buffer glycerine. Phosphate buffer glycerine (50% solution) Sol. No1: Disodium hydrogen phosphate-21.37 gms dissolve in 3,000 ml distilled water. Sol. No. 2: Potassium Dihydrogen phosphate -2.725 gms Dissolve it in 500 ml distilled water. Mix solution No.1 and 2 check pH to 7.4. Finally add 3500 ml of glycerine autoclave at 15 lb for 15 minutes. ii) In case of death, collect the affected part of the heart in buffered glycerine. iii) Small pieces from affected parts of organs including pieces of tongue, intestine and heart showing whitish grey areas preserved in 10% formalin. iv) Serum from the recovered animals (after 21 days of the disease.)
2.	Rinderpest and Mucosal disease complex	i) Defibrinated or citrated blood in OCG in febrile state (OCG supplied from the laboratory). ii) Serum samples, after 20 days of disease. iii) In case of death, piece of mesenteric lymph nodes and spleen in sterile vials sent on ice.

		iv) Small piece of various tissues showing lesions in intestine, lymph node, spleen in 10% formalin.
3.	Blue tongue	i) Blood from animals with fever collected in OCG , sent on ice ii)affected tissue in 50% buffered glycerine iii) Paired serum samples from acute and convalescent cased. iv) From autopsy, spleen and heart blood in sterile vials on ice and affected tissues in 10 % formalin.
4.	Infectious bovine rhinotracheitis	i) Serum samples collected on 0 day and 21 day of abortion or collected at acute of disease and after the recovery of respiratory infection. (Convalescent state) ii) Vaginal and nasal secretions for virus isolation. Detection collected with sterile brushed against wall of nasal passage and vagina during the acute phase of infection. Swab be sent in 2 ml transport medium containing Earle's Solution available from the laboratory. iii) Placenta, cotyledons and tissue from different parenchymatous organs of aborted foetus sent in the transport medium. All the materials in sterile vials sent on immediately special messenger. iv) In the absence of transport medium, tissue from organs of the foetus and cotyledons sent in sterile vials sent on ice with special messenger. v) Affected trachea and turbinate bones along with other organs showing lesions in 10% formalin, in case of death.
5.	Rabies	The brain divided in two longitudinal halves, half in formal saline. The other half in 50 % glycerine saline and sent through messenger to laboratory.

6.	Pox	i) Scabs in 50 % glycerine saline or unpreserved in sterile petridishes. ii) Scabs in formal saline. iii) Serum samples

Parasitic Diseases

S. No.	Name of disease	Material to be collected
1.	Theileriosis	i) Smears of the biopsy fluid obtained from prescapular or enlarged lymph nodes, fixed with methanol. ii) Thin blood smears air-dried and fixed with methanol. iii) In case the animal is dead, small pieces of enlarged lymph nodes spleen, uncleared part of abomasum in 10 % formalin.
2.	Babesiosis	i) Thin blood smears fixed in tight methanol. ii) From autopsy impression smears from heart blood spleen and kidney fixed with methanol and organs showing lesions in 10 % formalin, particularly spleen Urine from urinary bladder in clean test tube.
3.	Coccidiosis	i) Faeces and scrapings of intestine in 10 % formalin or 20% potassium dichromate solution. ii) Portion of affected part of intestine preserved in 10% formalin, along with other organs showing lesions for histopathology.
4.	Trypanosomiasis	i) Thick blood smears fixed with menthol. ii) 5 ml blood in vials having EDTA, Kept on ice. iii) if the animals is dead pieces of organs showing lesions, in 10% formalin, particularly liver and kidney.
5.	Mange	Scabs and deep skin scrapings in tight stoppered bottle.

6.	Trichomoniasis	i) Uterine discharge in a sterile vial, kept on ice. ii) Rest of the specimens collected as indicated under abortion.
7.	Anaplasmosis	Thin blood smears fixed in methanol.
8.	Gastro Intestinal Parasitism	Faecal samples in 5 % formalin saline.

Post- mortem Examination of Large Animals

STUDY OF GROSS SPECIMENS

STUDY OF GROSS SPECIMENS

Study of Histopathological Slides

AVIAN PATHOLOGY (UNIT-5)

POST MORTEM EXAMINATION OF POULTRY

Introduction

Systematic and thorough examination of carcasses for the study of gross pathological lesions with an objective to ascertain the cause of death is called as necropsy or post mortem examination. Itis one of the most valuable diagnostic techniques available.However, the first most importantcriteria of diseases diagnosis is the compilation of a good history before carrying out post mortem.Post mortem examination should be carried out at earliest because once purification sets in, then itbecomes tough to detect the tissue alteration and isolate the organism responsible for the disease. Iffresh dead birds are not available and the mortality continues and other clinical diagnostic methodsfail,few of the ailing birds may be sacrificed for detailed post mortem and laboratory investigation.

Materials required for a post-mortem examination

1. Small anlarg straight scissors (both pointed and blunt ends)
2. Curved scissors.
3. Small and large knives.
4. Bone cutter and a saw.
5. Scalpel or blade.
6. Bone cutter and a saw.
7. Small and largeforceps.
8. Hand lens.
9. Hand gloves-rubber orlatex.
10. Bunsen burner or spirit lamp or stove.
11. Spatulas
12. Syringes and needles
13. Sterilizer
14. Autoclave
15. Spirit or alcohol
16. Cotton and cotton swabs
17. Sterile swabs,vials,Petridishes and testtubes

18. Pasteur pipettes and rubber bulbs
19. Fixatives like10%formalin,formal saline or natural formalin
20. A microscope
21. Clean glass slides and coverslips
22. Different staining sets and stains like Gram’sZiehl-Neelsen,Giemsa and Wright’s strain
23. Normal saline and glycerin saline.
24. Small and large glass trays

25. Matchesandtorch
26. Aprons
27. Disinfectants like dettol,savlon,phenoletc
28. Insecticides or electricfly trap for controlling fly population.

Procedure

1. Soak the carcass in water andplace it on the necropsy table.
2. Keep the bird on its back with wings and legs extended. Abduct the legs by cutting the skin between the legand the abdomen one ach side and breaking open the hip joints and dislocating the head of eachf emur from acetabulum.
3. Cut the skin between the keel and the vent transversely and peel off the skin over the abdomen and legs.
4. Free the crop from the clavicular space by blunt dissection.
5. Cut open the abdomen by incising the abdominal wall transversely between the keel and the vent and remove the sternum by cutting through the ribs and clipping the corocoid and clavicles with bonecutter.
6. Examine the muscles of the thigh and sternum.
7. Examine the joints of the limbs.
8. Part the thigh muscles and examine the sciatic nerve with ventral side up.
9. Remove the sternum and expose the internal organs to study them insitu.

Examination of different organs

1. Remove the liver separately,avoiding damage to the gallbladder.
2. Remove the spleen and examine.
3. Remove the stomach and intestine in one piece afterc utting through the oesophagus just anterior to the proventriculus and tcloaca and examine the organs beneath.
4. Cut through the dorsal and ventra lattachment of oviduct with mesentery a nd removeit.
5. Remove the kidneys by blunt dissection and examine the sciatic plexus beneath.
6. Incise th e pericardialsac and observe for increased pericardial fluid.

7. Remove the heart by cutting the large blood vessels rising from the heart.
8. By means of blunt dissection free the lungs from the thoracic wall and cut through the dorsal attachment. Remove it by cutting the trachea just anterior to the syrinx.
9. Examine the brachial plexuses one ither side near the thoracic inlet along with the vagus nerve.
10. Expose the nasal cavity by cutting the upperbeak transversely near its attachment with the skin.

11. With the pointed end of the scissor, incise through the openings on either side to expose the paranasal sinuses and examine the abnormalities therein.
12. Open the mouth and cut from one angle of the jaw (preferably right side) with the blunt point of scissors and continue the incision through the pharynx down the esophagus andopen the crop.
13. Similarly open the trachea along its wholelength from the larynx and examine.
14. Examine the condition of the ribs for softness/hardness while cutting and assess the degree of bends in the long bones.
15. Section the proximal tibiotarsal and tasometatarsal epiphyses with a knife and examine for evidence of rachitic changes or abnormal structures.
16. Open and examine major joints.
17. Examine the surface of each visceral organ and incise for examination of cut surfaces.
18. Open hollow organs and examine its contents and inner surface.
19. Examine the brain by cutting and reflectin g the skin over the skull and the upper mandible.Carefully cut the skull and lift it to expose the brain.
20. Strip off the skin from the carcass and examine skeleton, bones,joints and muscles.
21. Record the lesions in the prescribed proforma.
22. After necropsy give a‘Pathologica diagnosis’ (Tentative/confirmative)based on the lesions encountered.
23. After identifying the etiology, give anetio-pathological diagnosis.

WRITINGOFPOST-MORTEMREPORT

REPORTONPOST-MORTEMEXAMINATIONOFPOULTRY

Species_____Colour_____Breed_____

Sex_____Age_____

Owner_____Residentof _____

Postmortem conducted by Dr._____

Assisted by_____

ByWhom sent f or Examination:_____

Date and hour of death _____

Date and hour of P.M. Examination_____

History:_____

External appearance (describes wounds, discharge etc.)

Interna appearance on removal of skin_____

Mouth and pharynx_____

Nasal cavities_____

Larynx and trachea_____

Oesophagusandcrop_____

Pleural cavity and lungs_____

Pericardium and heart_____

Peritoneal cavity_____

Liver_____

Spleen_____

Fore stomach stomach,abomasums_____

Proventriculus and gizzard_____

Smallintestine_____

Largeintestineandcaeca_____

Urinaryorgans_____

Genital organs_____

Brain and spinalcord_____

Bones and joints_____

Muscles and tendons_____

Lymphnodes in general_____

Endocrine glands _____

DIAGNOSIS

Additional Examination needed

PostMortem done by_____

Date_____

COLLECTION OF MATERIALS FOR LABORATORYEXAMINATION

In general the materials are collected in 50% GPBS (Glycerol Phosphate Buffer Saline) for virological investigation & in 10% BNF (Buffered Normal Formalin) for histopathology.

S.NO	DISEASE	MATERIALS TO BE COLLECTED
1.	New Castle Disease	Brain, Bone marrow, Lung, Liver, Spleen & serum samples.
2.	Infectious Bursal Disease	Bursa of Fabricius , Spleen, Kidney, & Liver.
3.	Marek's Disease	Brain, Nerve, Ovary, Testis, Liver, Spleen, & Feather follicle(For Virology).
4.	Lymphiod Leucosis	Liver, Spleen, kidney, & Gonads.
5.	Infectious Bronchitis	Trachea, Lungs, Airsac, Kidney (for virology).
6.	Infectious Laryngo Tracheitis	Tracheal exudates for serology& virology. Trachea For histopathology.
7.	Avian Encephalomyelitis	Brain for virology, serum samples, Brain,Pancreas & proventriculus for histopathology.
8.	Avian influenza	Tracheal plug, Brain, & Pancreas for virology. Brain, Pancreas, Liver, Spleen & Lungs for histopathology.
9.	Inclusion Body Hepatitis	Liverforvirology &histopathology.
10.	Egg Drop Syndrome	Affected eggs for virology & serumsamples.
11.	Fowl Pox	Pieces from skin & other tissue lesions,serum samples.
12.	Leechi Disease	Liver for virology &histopathology.
13.	Chicken Infectious Anaemia	Liver impression smears, Thymus,bonemarrow,& Liver for virology.
14.	Colibacillosis	Heart, Liver,&lungsfor bacteriology.
15.	Infectious coryza	Swabs from infraorbital sinus, trachea, & Airsac for bacteriology.Serumsample.
16.	Fowl Cholera	Impression smear from liver&lungs,heart bloodsmear, Liver,Bonemarrow,Heartblood for bacteriology.
17.	Fowl Typhoid	Liver,Spleen,&Ceacaforbacteriology,Whole

		blood&Serumforserology.
18.	Pullorum Disease	Liver,Spleen,&Ceacaforbacteriology,Whole blood&Serumforserology.
19.	Necrotic Enteritis	Intestinal contents,Scrapingofintestinalwall, hemorrhagiclymphoid nodulesfor bacteriology
20.	Gangrenous Dermatitis	Exudatesofskin&subcutaneoustissueor underlying muscleforbacteriology
21.	Tuberculosis	CrushedlesionfromLiver,Spleen&bonemarrow For bacteriology, Wholeblood forserology
22.	Spirochaetosis	Blood smear s o rstaining,wetbloodfilmfordark Field microscopy. Serumsamples.
23.	Chronic Respiratory Disease	Swabsfromnasalcavity,Airsacs,Trachea,&lungs,exudates aspirated from infraorbital sinus&joints For bacteriology
24.	Aspergillosis	Smears from lesions, & lesions for culture.
25.	Mycotoxicosis	500gm of suspected feed, &mouldyclumps
26.	Chlamydiosis	ImpressionsmearfromsurfaceofLiverorSpleen&Cloacal,Trachealorcojuctivalswabsforculture. serumsamplesforserology.
27.	Coccidiosis	Sickbirds&feacesforexamiation,Pieceof intestine&caecum for histopathology

Study of gross specimens

Study of histopathological slides

Diagram of Internal Organs of Poultry

Diagram of Digestive System of Poultry

UNIT-6 (PATHOLOGY OF DISEASES OF LABORATORY AND WILD ANIMALS)

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Post mortem Examination of Zoo and Wild Animals

Preparation for Post-mortem examination

When mortality or morbidity in a wildlife population is reported, the veterinarian must collect as much general and local information as possible, including any evidence of similar mortality or morbidity in animals. A thorough inspection of the surroundings of a sick or dead animal is important.

Care should be taken for signs of a struggle which may indicate an encounter with a predator at the time of death. An accumulation of faeces behind the carcass may indicate a period of immobility, as may heavy browsing of food plants within easy reach. If the animal is found alive, it should be observed from a distance and note taken of any peculiarities of gait, respiration, excretion and unusual behaviour.

Photographs may also be of great value if litigation is likely. The performance of a thorough post-mortem examination plays an important part in the diagnosis of the cause of death.

Notifiable diseases

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

Notifiable diseases, which include rinderpest, foot and mouth disease, classical swine fever (hog cholera), tuberculosis and Newcastle disease, are those which are of great severity, have a high ability to spread rapidly or are of socio-economic and/or public health importance. These diseases are governed by strict national and international regulations.

Post- mortem site

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

A pit may have to be dug to dispose of the carcass, so a site where this is feasible should be chosen.

Safety precautions

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

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

Anthrax is a common cause of sudden death in ungulates and carnivores which prey upon them. Carnivores, omnivores and insectivorous bats may have died of rabies and carnivores may be infected with the small tapeworm of *Echinococcus* spp. Tuberculosis is an emerging disease in some wildlife populations and brucellosis can infect wild herbivores.

In view of the above, any person conducting an autopsy on any wild animal should be aware of the risks involved and should wear appropriate protective clothings. Face masks, which cover the eyes, nose and mouth, are particularly important when examining animals suspected of infections likely to become air-borne during dissection. Great care must be taken to ensure that all specimens taken from a carcass are collected, stored and transported safely and that there is no risk of the escape of infective material.

Post-mortem procedure

This consists of two parts: -

(a) Ante- mortem examination (b) Post- mortem examination

(a) Ante- mortem examination: - It includes brief history of case, clinical signs and symptoms shown by the wild animal, tentative diagnosis and line of treatment, reports of the examination of blood, stool, urine etc.

(b) Post- mortem examination: - This consists of two parts:

1. External exam. 2. Internal exam.

1. External exam.

(i) Identification of the wild animal on the basis of age, body markings etc.

(ii) Body coat of the animal

(iii) General condition of the carcass

(iv) Any discolouration on the body

(v) Injury and wounds

(vi) Tumours on the body

(vii) Any fracture and dislocation

(viii) Examination of the natural orifices

(ix) Condition of eye and pupil

2. Internal Examination-Necropsy should be conducted in a systematic manner. It is, therefore, essential to assign specific task to each of the team members. A team consisting of two or three assistants may be assigned to each of the following: (a) Head (b) Fore-limbs (c) Hind-limbs (d) Thoracic region (e) Abdominal region (f)

(a) Head: Disarticulate the head while cutting through the atlanto-occipital joints and separate from the body. A good portion of the cranium must be cut to reach the brain. Remove the bony plates by lifting them with a crowbar and expose the brain. The brain is dissected out after severing the attachments.

(b) Fore-limbs: Disarticulate and remove the segments after cutting through the shoulder joint, elbow joint, the radio-ulnar and carpal joints.

(c) Hind-limbs: Remove the segments as above, after cutting through the hip joint, stifle joint, tibiofibular and tarsal joints.

(d) Thoracic cavity: Dissect out the skin and subcutaneous tissue along the vertebral column and lift them up. Put an incision in the middle of the dissected skin; pass a rope and tie. Pulling the rope can flap the dissected structures. Separate and remove the structures by making deep incisions at the margins of the ribs at the cranial and abdominal ends of the thoracic wall and connecting them with a deep ventral mid-line incision. Cut the vertebral articulation and expose the thoracic cavity by pulling the ribs along with the muscles as above and severing it from the costo-chondral articulations of the sternum. The heart, lungs and associated structures are thus exposed and can be cut and separated.

(e) Abdominal cavity: Dissect out the skin, subcutaneous tissue and muscles by making deep incisions along the ventral mid-line from the xiphoid to the pubis. Lift up the structures and separate them out by putting deep incisions from pubis to the anterior border

of the iliac crest and along the lumbar vertebrae. Slip off the omentum and pull out the intestine and stomach. Remove the liver and spleen separately after dissecting their attachments. The kidneys, bladder and the reproductive organs (uterus and testes) are removed by separating their attachments.

(f) Examination of organs All parts of an organ should be examined thoroughly. Emphasis should be given to differentiate lesions from post-mortem changes.

Post-mortem Report:

Institution /Owner:
Address:
Post-mortem No.:
Species:_____ Age:_____Sex:_____Colour:_____
Wild_____ Wild caught_____ Captive born_____
Date and time of death:
Death location:
Necropsy date: Time:
Necropsy location:

Post-mortem diagnosis:

Etiopathological diagnosis: (this can be done after evaluating the postmortem lesions and the report of laboratory examination of materials being collected and sent during necropsy)

Post-mortem done by:

Date: _____

Protective clothing

A list of protective clothing is given below:

– rubber boots

– rubber or plastic gloves

– rubber apron

– overalls

– face mask including goggles to cover eyes.

Post-mortem equipments

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

– curved knife for skinning
– straight, pointed knife for dissection
– Pointed forceps
– Dissecting scissors
– sterile scalpel and blades
– bone saw
– large pair of bone forceps or bone-cutting shears
– axe
– sharpening stone and steel
– spring balance
– some nylon rope
– small gas or spirit burner for sterilising instruments.

The kit may be packed in a wooden box.

Specimen containers and sampling equipments

– sterile disposable 5 ml syringes and sterile needles
– culture tubes with sterile swabs
– microscope slides in box
– sterile Universal bottles
– sterile blood tubes
– plastic bags with closure tops (Whirlpack or Ziploc type)
– plastic sealing tape
– plastic jars
– measuring tape or ruler
– rubber gloves
– aluminium foil
– labels, waterproof marker pen/pencil

Transport equipments

For transportation, the following equipment is required:

– an insulated, plastic cooler box

– a leak-proof, screw cap, plastic containers

– absorptive packing material

– plastic sealing tape

– 50% glycerine

– ice packs

Fixatives

The following fixatives are used:

– 10% formalin

– 70% alcohol for parasites

Disinfection materials

Disinfection materials include the following:

– plastic bucket and brush

– soap and towel

– borax

– 5% formalin

– 70% ethyl alcohol for disinfecting instruments.

Study of gross specimen

Study of histopathological slides of diseases

Students notes