Collection, Preservation And Shipment Of Specimens For Laboratory Diagnosis

S. Nandakumar

Introduction

Since time immemorial, man had been rearing livestock as evidenced by the ancient Hindu and Christian mythology, where Lord Krishna and Jesus Christ were into it, though not in its truest sense. This industry showed leaps and bounds in its development and it can be noticed that except fora few seasonal fluctuations, there has always been a steady incline in the growth pattern of this sector over the years. But in recent times, it is an imperative question whether to continue or forbid this tradition which our ancestors have set in. this can be attributed to the socio-economic changes taking place in the rural sector as a result of the high expenses incurred in maintaining the animals, the minimal profits received in return and the prohibitive cost of treatment procedures. Even in the wake of such situations, majority of the people in our country resorts to Animal Husbandry activities owing to the cost-effectiveness of this occupation and the everlasting demand for livestock products in our country. There is a high incidence of newly emerging diseases, rarely diagnosed in the early stage, which poses a serious threat to the livestock sector in the state. This is further alarming when we consider the financial constraints faced by the marginal farmers to make both ends meet through this enterprise. Disease diagnosis had only limited value in age-old days. But of late, much emphasis is given to this aspect by the new generation veterinarians so as to improve the quality of service rendered by them.

Disease if diagnosed at an early stage helps the veterinarian to have the right shot at the right target without waiting for the worst to happen. The skill of a clinician is equally important or even more than a laboratory diagnostician with regard to disease diagnosis. Based on the history of the case and the facts drawn from clinical examination, the clinician arrives at a conclusion – "tentative diagnosis" and to confirm his findings, he collects appropriate samples, preserves it and sends it to the laboratory for confirmatory diagnosis.

Dr. S.Nandakumar MVSc.
Veterinary Surgeon,
Chief Disease
Investigation Office,
Palode.

Collection of specimens

The results of any laboratory examination depend on the selection, mode of collection, preservation and transportation of the specimen. A representative sample should be collected form a typical case of infection. In the event of an outbreak, an animal with typical signs of illness should be sacrificed for sending to the laboratory. Clinical samples from such animals should be collected at the peak of the disease. A complete postmortem examination should be done taking into account each and every system and the gross changes in the individual organs are noted for future reference and to correlate with the findings of subsequent examination. It is highly essential that the clinical samples should be collected before the start of the medication. A wide rage of tissues should be collected to avoid changes of failure to detect the pathogenic organism involved. The details showing the history, clinical symptoms and tentative diagnosis. Should accompany the specimen sent to the laboratory for examination. Specimens get spoiled due to numerous causes-

a. Haemolysis

due to wet syringe/needle, forceful discharge through needle, un due agitation, un due heat or cold, bacterial decomposition, chemical contaminants etc.

b.Bacterial decompositions

due to contamination with fecal/ intestinal/material, high temperature and prolonged period of transit.

c. Autolysis

due to dry packing with antiseptics, high temperature and prolonged period

of transit.

d. Dessication

due to small volume of sample or non-airtight container that permits evaporation.

e. Fragmentation

due to dull knife/scissors in cutting, forcing the specimen in small bottles, insufficient or improper dehydrating and fixing preservatives during transit and freezing the specimen with dry ice or in a deep freeze unit.

Blood

For collection of whole blood, venepuncture with a needle and syringe is performed on any one of the prominent superficial veins. The sites of collection are jugular vein for horse, cow, sheep and goat, radial/saphenous veins for cat and dog. Rabbits, mink, guinea pigs etc., are most easily bled from the heart or from the marginal ear vein by nicking it with a No.II Bard Parker blade. Pigs are bled from the anterior vanacara or ear vein or amputation of tail. Heller and Paul's double oxalate mixture, sodium citrate, sodium oxalate, ekhylene diamine fetra acetate and Heparin are the commonly used anticoagulants. Total erythrocyte and lencocyte counts can be delayed upto 24 hours without serious errors, provided the blood is kept in cold. Whole blood should not be frozen. If the whole blood is used for determining the Erythrocyte Sedimentation Rate (ESR) the test should be conducted within 1 to 2 hours. For bacteriological examination, blood should be collected aseptically after cleaning the area with 70% alcohol. Blood can be inoculated directly into thioglycollate broth or Hartly's broth or sent to the laboratory. For diagnosis of Leptopirosis, blood should be collected during the first week of illness. Blood smears can be prepared within 15 minutes of collection by slide method or cover glass method. The smears are best stained within one hour of preparation. If the slides can't be stained within a reasonable time it can be preserved. For diagnosis of Dicrofilariasis, a drop of blood placed or glass slide is covered with cover slip and examined under microscope (wet film examination).

Urine

A chemically clean container should be used. The sample may be collected while the animal is urinating or by catheterisation. The latter method is more preferred because there will be less uretheral or vaginal detritus in such sample. Midstream urine is usually collected. Pressure can be applied to the bladder to express urine in cats and dogs. Collect at least four ounces of urine. For bacteriological examination, urine is aseptically collected by catherisation and inoculated into media before or after centrifugation. For the diagnosis of Leptopirosis, urine should be collected towards the end of second week upto 40 days. For fungal examination morning samples are best because the organisms grow over night assuming their identification. For microscopic examination of urine to detect urinary sediments, the sample must be centrifuges.

Milk

For collection of milk sample, the first two as three strips of milk should be avoided. For bacteriologic examination, milk is collected aseptically. The teat orifice is cleaned with 70% alcohol or iodophor lotion. The milk from the affected quarters is collected in sterile plastic/glass vials and labelled. The milk should be properly mixed before starting the test. For mastitis diagnosis, the california mastitis test and strip cup test is not reliable at the beginning or near the end of lactation. For diagnosis of coliform mastitis, two or three consecutive samples may be required for getting a positive isolation.

Faeces

Collected a fresh specimen free from soil, stones etc., into small glass jars. Waxed paper can be used where as absorbent/tissue/newsprint paper is not satisfactory. Faeces collected and allowed to remain at room temperature for a few hours will contain eggs whose embryos are much more fully developed. For diagnosis of lungworm infection, freshly voided faeces are preferred. Rectal swabs are collected and cultured aerobically and anaerobially for bacterial isolation.

Skin scrapings

Ectoparasites like ticks, lice and fleas are relatively easy to recognise as they are macroscopic in size and have typical structural characteristics. Skin scrapings are done for the detection of mites. Lesions recently developed are more suited than old lesions. The periphery of the lesion is a richer source than centre. The scrapings should be made from the deeper layers of the dermis with a dull scalped to produce pin point haemorrhages. This assures one of reaching the papillary stratum. When demodectic mange is suspected, use pressure to express the mites from the hair follicles. Dampen the area to be scraped with water or physiological saline, so that the material adheres to the scalpel. The scalpel used for collection can be dipped in mineral oil. The oil clarifies the serum scales and other debris and makes examination easier. It also prevents desiccation of the sample. If the same sample is used for fungeal examination also, it's better not to use mineral oil. When the lesions are vesicular, remove the roof of the vesicles with a scalpet blade and include it as a specimen skin can also be collected by scotch tape method.

Cerebrospinal Fluid

Collect c.s.f. from the cisterna magna at atlanto-occipital articulation/lumbar function. A minimum quantity of 1ml is required. The cell count and examination for glucose and bacteria should be performed at once. For bacteriological examination, the collection procedure should be sterile.

Exudates from eyes, ear and nose

The exudates may be collected for bacteriological examination using dry sterile cotton swabs. For collection of specimens from nose and ears, the swab is introduced into the nose/ears as far as possible. Then it is gently rotated and the swab is placed in transport medium and the extra length of the swab broken off and the bottle is capped. The material can also be directly inoculated in solid media and cultured in clean dry vials as smears can be prepared on glass slide for the diagnosis 12 of nasal schistosomiasis, Rhinospordiosis and Carcinoma.

Pus and exudates from wounds

Before collection, disinfect the external; surface using 70% of alcohol. Pus samples or discharges from septic wounds are collected in sterile tubes for bacteriological examination. The swabs can be rolled in glass slider for preparation of smears. Pus/exudate from sinus tracts, draining fistulas or unopened areas should be collected aseptically for mycological examination. Pus/exudate collected by swabs will not be sufficient for mycological work so aspiration is usually performed. Pustules are squeezed between the thumb and finger of the left hand thus expelling the contents. This can be maculated into culture media for bacterial isolation.

Genital discharge

Specimens are to be collected from the cervix and uterus. Special care should be taken to avoid contamination with vaginal flora. A uterine biopsy catheter or a three-way catheter can be used to collect uterine mucous-membrane and uterine contents/uterine washings. The

uterine pus is a rich source of trichomonas so it can be siphoned pout with a clean catheter. Vaginal pus if it is scanty can be mixed with physiological saline or a buffer solution. Cervical and vaginal swabs can be collected. In case of male animals perpetual washings and semen can be collected aseptically. Perpetual swabbing may be collected by swabbing forcibly with a cotton peldget held with a forceps or the material may be aspirated from the prepuce from the space around the glans by means of a pipette.

Serum samples

Serum samples from affected/incontact animals are frequently required to permit a diagnosis. Dry syringe and needle must be used for collecting blood. No anticoagulant should be added. Usually paired serum samples are collected. One collected during the acnte phase of illness which provides a baseline to compare the antibody level and a second sample collected during the convalescent phase. Approximately 14-21 days. To collect serum, first draw blood by venepuncture doesn't freeze. Allow it to clot in a tube at an angle and the clot is released from the wall by jarring it against the palm or with a splinter. Then incubate the tube at 37° C for 30 minutes and then placed in refrigerator. The clot retraction usually expresses sufficient serum in 12+18 hours. If the serum is scanty, centrifuge at 1000 r.p.m. for 10 minutes.

Morbid specimens/tissues

Pieces of various tissues and organs collected by biopsy, surgery or at autopsy can be used for direct microscopical examination, cultural examination, animal inoculations and histopathological examination. For histo pathological examination slices of tissue cut should include the gross lesions and should show sufficient adjoining normal tissue to identify the tissue and show the nature of spread. Make sure that the specimens reach the laboratory within the minimum time possible. Pieces of lung in cases of Aspergillosis impression smears of various organs etc. can be collected for microscopical and histo pathological examination. Large number of intestinal bacteria invade the body before and after death.

So specimens collected on autopsy long after death doesn't usually reveal the causative organism. Collect pieces of internal organs depending on the gross lesions noticed. Uterine discharge, pieces of placenta, aborted fetus/fetal stomach contents, plenral/peritoneal fluid, fetal liver, spleen or heart can be collected in gloss/plastic containers sent to the laboratory. Intestinal contents can be collected in polythene bags in cases of enteric infections. If the carcass is decomposed, bacterial organisms can be isolated from the bone marrow of long bones. In suspected cases of anthrax, muzzle pieces/ear tip can be sent to the laboratory. In poultry, samples from moist membranes such as trachea, sinuses, airsacs and soft organs. Such as liver, spleen, brain and kidney can be collected on sterile cotton swabs. Culture can be done directly from these swabs, or else in case of delay, the swabs should be placed in nutrient broth to prevent desiccation.

Preservation of specimens

To obtain satisfactory results from laboratory diagnosis in addition to the correct sampling, appropriate preservation, careful packing and proper shipment of the specimens are essential., great care should be taken in the preparation of specimens so as to avoid any deterioration or damage to the sample. Material for microbiological examination should reach the laboratory within six hours of collection. But if the materials are kept at 2-10°c, the transit time can be

extended upto 18 hours. The two methods of preservation are veterinarian and chemical preservation. Refrigeration is the most effective method. This is extremely useful for sending animal carcasses for anatomic and pathologic studies. Natural ice may be used safely. Some absorbent material like sawdust is included to absorb the water from the melting ice. Dry ice can be used but it is not easily available. Dry ice can preserves the specimen for two to three days. Refrigeration before shipment is a good practice in cases of blood samples for Brucella diagnosis, when samples are sent during hot weather. Place the sample in refrigerator overnight. Arrange bleeding so that the samples reach the laboratory early in the week.

Chemical preservatives may be classed according to their effects. Each class has a specific place as a preservative.

a.Dehydrating and fixing solutions for preservation of specimens for histopathologic studies. Formalin solution (10%) – approximately 4% formaldehyde gas is the commonly used chemical preservative for preservation of tissues/organs obtained on autopsy. The volume of the preservative should be ten times the volume of the tissue to be fixed. Zenker's fixative and klott's solution if available can be used. Absolute of 90% alcohol can be used as an alternative to formulation, but interior to the latter.

Rubbing alcohol (50-70%) should not be used, since it hardens and dehydrates the tissue. Blood smears can be fixed in methoano for transport to laboratory.

b. Bacteria inhibiting preservatives are used when the bacteria are to be kept at minimum. Powdered Boric acid/ Borax can be used for short time the preservation. But this is not too desirable due to the dehydrating effect on tissues. Boric acid will not prevent decomposition if the specimen is in contact with the intestinal tract or soiled by faecal/intestinal material. Neutral glycerin 50-100% can be used for virus isolation. Since viruses are sensitive to changes in pH_1 50% glycerol saline pH 7.4 is preferable for preservation of tissues of virus isolation.

c. Bacterial solutions mainly used for serum preservation. Merthiolate (aqneous) 1:10,000 concentrate or phenol –0.5% can be used for preservation of serum samples. Faecal samples can be preserved using 10% formalin. Toluol stratified on a urine sample is an adequate preservative. 2-4 drops of 10% formation/ounce of urine is another preservative. Boric acid at the rate of 0.5g/28ml of urine prevents bacterial multiplication for four days.

For hydrocyanic acid detection, rumen contents and liver pieces should be detection of ectoparastici infestations could be preserved in mineral oil. Vaginal/uterine material and perpetual swabs can be flushed with physiological saline and preserved. Cervical and vaginal swabs are placed in Leiboritz medium/surcose solution/nutrient broth for bacteria and virus preservation. Parasites collected from intestinal contents or other means can be preserved in physiological saline solution. Parasites can be killed and preserved by using 10% of formation or 70% alcohol containing 5% glycerine. Oxlsated blood preserved in ice can be used for the detection of blood protozoan like Anaplasma, Babesia, Incileria and Leptospira., unstained blood smears used for the detection of the above organisms can be preserved by fixing in methanol. Bone marrow aspirates are preserved in sterile containers containing distilled water. Exudate from dyes, nose and ears should be preserved in transport medium for virus isolation. Specimens collected by swabs dry out fast. So it world be better if two slides are

prepared and sent along the swab to the laboratory. Take care to see that neither the slide stickis to each other not the smear being wiped off during packing. For virus isolation, the following transport media can be used-phosphate glycerine saline (1:1), simple glycerine saline (1:1), commercially available transport medium, Hank's Balanced Salt Solution and Tryptose phosphate broth. Preservatives previously mentioned or antibiotics like penicillin loo IU and streptomycin 100 mg/ml of serum can be used for preservation of virus suspected serum samples/other samples. Serum samples should be heated to 56°c for 30 minutes to destroy the non-specific reactions. Serum should be stored at –20°c in screw capoped vials. Lymphnodes/ nodules well refrigerated impression smears can be used for the diagnosis of tuberculosis. Specimens of tumours of small size or biopsy material should be preserved in as such in 10% formation. In large tumours and diseased organs., small pieces ¼ x ¾ x ¾ inches size or lesions can be preserved in 10% formalin/ formol saline. Milk samples can be preserved by refrigeration.

Shipment of specimens

Immediately after collection and preservation. Place the specimen in suitable labelled containers that are air tight, leak proof and resistant to breakage. Add appropriate transport medium. The medium should be sufficient to withstand any likely evaporation, but should not be so large resulting in unnecessary dilution of the specimen. It must be buffered to avoid extremes of pH. It would be better if some stabilising agents like bovine serum albumin are added which will keep the ineffectivety of viruses and also buffer the medium. Specimens collected in small amount like swabs and lesion scrapings should be immediately transferred to transport medium for shipment. Shipment in natural ice follows the process detailed below. place the specimen in water tight container which is agina kept in a larger container. The space between the two containers is tilled with cracked ice. Saw dust may be filled so as to absorb the water from the melting ice. Make sure that the containers are properly sealed. All the containers must be labelled. Plastic picnic bags, wooden cartons/boxes or large containers can be used for shipment. Shipment of specimens can be any of the following methods – Parcel post, Railway or bus. If possible it is advisable to send a special messenger along the specimen.

When anthrax suspected material is sent to the laboratory mark "Anthrax suspect" on the container to alert the laboratory personnel to potential danger. For rabies suspected carcass, the animal as a whole or at least the head should be placed in an entirely closed watertight container. Pack ice and sawdust but don't freeze. Don't add chemical preservatives. Place two addressed tags and a label indicating that the package contains the head of an animal suspected of having died or rabies. Include the following information in the request, which should accompany the specimen.

1. Name and address of the persons submitting the specimen
2. Name and address of the owner of the animal
3. Name and address of the anyone who may have been bitten
4. Location of the bite
5. Name of the physician
6. Was the animal vaccinated for rabies?
7. Was there contact between this animal and other animals

Information to be submitted to the laboratory with any clinical material specimen should include more than just

Continued on page 50