Clinical pathology (L2)

Dr. / Muntadher M.

Preservation of samples:

1. fecal : preserved in 10% formalin to minimize development and hatching

٢ . Blood : (Anti-clotting tubes)

٣. Urine : o.o1% merthiolyte

4. Nasal Discharge : In test tube containing **5 ml of 10% KOH or 5% NaOH** .

Unnecessary agitation of the nasal discharge has to be avoided

5. VAGINAL DISCHARGE :

Clean the external genitalia thoroughly before taking .samples in order to avoid contamination with intestinal protozoa

Take samples from the vagina by introducing about **10 ml physiologic NaCl solution with a bulbed syringe and washing it back and forth several times by squeezing the bulb repeatedly**

Preservation •

Vaginal discharge should be examined on the same day, hence preservation is not advised as the organism lose its motility and morphology

PARASITES / SEGMENTS OF HELMINTHS

- Collection

Collect the helminths during postmortem examination, sometimes immature amphistomes and segments of tape worms can be collected from dung or faeces.

- Preservation

- *Trematodes:* Fresh and live flukes are collected in normal saline. 70% or 90% ethyl alcohol used as a preservative and fixative.

- *Nematodes:* Nematodes are fixed in 5-10% hot formalin or 70-90% ethyl alcohol. Once the nematodes are fixed they can be stored indefinitely in glycerin alcohol.

- *Cestodes:* Tapeworms including the scolices (heads) should be placed in water at about 37°C for about one hour and then stored in a mixture of 5% glycerine and 70% alcohol or 5-10% formalin.

Feces culture :

It is used for detection the presence of helminthes ova and ensuring their incubation, and the development of larvae to the infective third stage.

Procedure of feces culture :

1- The feces broken up and placed in a glass jar.

2- Glass jar is closed and kept at a temperature of about 26˚c for a suitable time, usually 7 days .

3- After incubation, concentrate the larvae by means of the Baermann technique .

Procedure of Baermann technique : (used of the larvae diagnosis)

1- Apply ± 20 g of fresh feces to a gauze.

2- Fill the funnel with tap water ,so that the feces are completely immersed .

3- Let the whole settle at room temperature for 24 hours. The larvae will come out of the soaked feces and fall through the meshes into the funnel neck where they are concentrated at the bottom .

4- Release the clip and collect the first 3-4 drops on a microscopic slide, or collect 5-10 ml of the liquid, which may be drained off into a centrifuge tube where the larvae will be in the sediment .

5- Examine the nematode larvae under low power without cover glass. The larvae will be swimming actively .

Note :

In heavy infestation, larvae can be drawn off in a drop of water after an hour, but when few larvae are present, it may be necessary to leave the Baermann set up overnight .

Adhesive-tape method :

This method used in horses for the determination of *Oxyuris equi* because their eggs stick to the anal region and

usually are not found in the feces. The method use a transparent **adhesive tape 2.5 cm wide and ± 15 cm long** .

1- Clean the area surrounding the anus day before the sample is to be taken .

2- Stick the tape onto the right thumb, and firmly press the adhesive tape to the anal skin folds .

3- The adhesive tape is stuck on to a microscope slide .

4- To examine the preparation, a drop of water is placed under the tape .

5- The strip is then again firmly stuck and the preparation is examined under the microscope .

Quantitative Methods :

To obtain more accurate information with regard to the severity of an infection, egg counting methods have been devised. There are two methods can be used: Stoll's dilution method and McMaster method .

McMaster egg-counting technique :

1. Weigh 2 g of feces accurately .

2- Soaked it in a 60 ml of saturated solution ,then strain the solution through a fine sieve .

3- Fill the compartment of the counting cell in the McMaster slide .

4- After a few minutes the eggs float up to the surface of McMaster slide and they are all in the focus against the upper slide.

5- Count the eggs at least in two compartment using a X 10 objective ,according to the following calculation :

$$\frac{\text{Total number of eggs}}{\text{Number of chamber}} \times ٢٠٠ = (\ ) \text{ EPG}$$

** Copra test : depended Ag XAb in fecal