	NATIONAL CENTRE FOR ANIMAL HEALTH **LABORATORY SERVICES UNIT**	**Second edition**
	STANDARD OPERATING PROCEDURE	**Version 2018.1**
	POSTMORTEM	

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TITLE: Parasitology sample collection

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

A. FAECAL SAMPLE COLLECTION

1. **Purpose:**

The purpose of this SOP is to describe the procedure for collection of faecal samples from animals.

2. **General information:**

Eggs and larvae produced by adult helminths in the host animal are often passed out in the faeces. The demonstration of eggs or larvae in faeces can indicate the presence of parasitic infection and facilitate the diagnosis of parasitic disease. The eggs and larvae can be identified and quantified.

3. Equipment/materials:

3.1 Rubber gloves
3.2 Marker pen
3.3 Faecal vials (Scintillation vials-8ml and 20ml plastic vials)
3.4 Laboratory submission forms
3.5 Spatula

4. Reagents, solution and buffer

4.1 10% formalin

5. Procedure:

5.1 About 30 g of freshly voided faeces should be should be collected in the container and be sent to the laboratory within 24 hours. If transport times are likely to be longer than 24 hours, the sample should be sent on ice or refrigerated or preserved in 10% formalin before sending to the laboratory in order to avoid decay and hatching of larvae.

5.2 The faeces should be collected fresh either from the rectum or from the recently defecated patch. The jar should be filled and the lid closed tightly. An alternative method is to take swabs from the rectum (or cloaca), taking care to swab the mucosal surface.

5.3 The swabs should be visibly coated with faecal material; however, samples collected with a swab are inadequate for parasitology. Care should be taken when

collecting swabs from small, delicate animals or birds to avoid injury to the animal; small swabs are commercially available that should be used.

5.4 Swabs should be transported in appropriate transport medium and are best stored and transported at 4°C. Sample should be collected from normal as well as sick animals.

6. Safety

Outmost care should be taken to avoid the infection to the collector. If the feces contain an infectious organisms like Coccidia, giardia and Toxoplasma etc. Can be infectious to people.

Formalin is irritant and toxic: use with care in a well ventilated space and avoid inhaling fumes.

7. Trouble shoot

For a valid analysis, the feces should be submitted to the laboratory within 24 hours of being passed by the animals, and preferably within 12 hours. If this is not possible, the sample should be kept in a cool area (but not frozen) out of direct sunlight or use 10% Formalin as preservative.

8. References

- Clinical Veterinary Advisor, 2nd Ed. 2011
- RVC/FAO guide to Veterinary Diagnostic Parasitology

9. Appendix

Preparation of preservative :(10% formalin)

Formaldehyde – 5ml

Distilled water – 95ml

B. ADULT WORMS AND CYST SAMPLE

1. Purpose:

The SOP is aimed to outline the procedures for collection of adult worm and cyst samples.

2. General information/responsibility:

A number of parasites will be found in almost every grazing animal, irrespective of the state of its health. To assess the significance of parasite infections in field mortalities, it is therefore necessary not only to determine the species present, but also to assess the number of each species.

3. Equipment/materials:

3.1 Petri dish
3.2 Rubber gloves
3.3 Marker pen
3.4 Spatula

4. Reagents, solution and buffer

4.1 0.85 % NaCl solution
4.2 70% alcohol containing 5% glycerine
4.3 10 % formalin solution

5. Procedure:

5.1 Nematodes:

5.1.1 Collect worms into tube/ petri dish containing 0.85 % NaCl solution.
5.1.2 Shake gently to remove debris.
5.1.3 Transfer worms into hot 70% alcohol. (It fixes the worms in stretched position for easier identification)
5.1.4 Store in 70% alcohol containing 5% glycerine or helminth preservative fluid.

5.2 Trematodes:

5.2.1 Collect the worms in Petri-dish and shake vigorously for 1 min in 1% Sodium chloride.
5.2.2 Add equal quantity of saturated mercuric chloride solution and allow it to stand for 10 minutes. (This kills the worm in an out stretched position).
5.2.3 Wash properly in running water.
5.2.4 Store in 70 % alcohol, 5% glycerine added or helminth preservative fluid.

5.3 Cestodes

5.3.1 Collect worms in petri dish and relax worm in water bath at 40°C for about 15 to 30 min.
5.3.2 Drop in fixative. (Example-10 % formalin solution or helminth preservative fluid). This will preserve worm in stretched position.
5.3.3 Flukes, tapeworms and roundworms should be washed in water and preserved in 5% formalin. Always include the head of the tapeworm. When identification of parasitic cyst is required, tissues should be submitted chilled in a plastic bag or jar.

6. Safety

- N/A

7. Trouble shoot

- N/A

8. References

- N/A

C. BLOOD PROTOZOAN PARASITES COLLECTION AND PREPARATION

1. Purpose:

For confirmatory diagnosis and it is highly essential to provide efficient treatment and effective control & preventive measures.

1.1 To carry out the surveillance or prevalence of economically important diseases.
1.2 Monitor the health of the animals.
1.3 For health certification of animals for both export and import purposes

2. General information/responsibility:

A blood film or peripheral blood smear is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically. Blood films are usually examined to investigate haematological problems (disorders of the blood) and, occasionally, to look for parasite within the blood such as *Babesia*, *Theileria* and *Trypanosomes*.

3. Equipment/materials:

3.1 Rubber gloves
3.2 Marker pen
3.3 Laboratory submission forms
3.4 Syringes,
3.5 Hypodermic needles (for collection of blood),
3.6 Glass slide
3.7 Vacutainer EDTA (Purple cap), Heparin (Green cap).
3.8 Razor blade
3.9 Alcohol swab

4. Procedure:

4.1 **Thick smear preparation**

4.1.1 Take two grease free glass slides
4.1.2 On the edge of the one slide place a drop of blood either collected fresh from the blood collected in EDTA vial.
4.1.3 Now using the edge of the other slide, gently make a thick smear of blood by stirring the edge of the slide in a circular fashion.
4.1.4 Let the smear air dry
4.1.5 If you need to dehaemoglobinize your smear, after air drying gently dip the smear in the water till u see the slide become clear.

4.2 **Thin smear preparation**

4.2.1 Prepare spreaders. Score a slide with a glasscutter or with a diamond pencil. One slide should make about 4 spreaders. The spreader should be 3-4 mm narrower than the slide on which the blood film is to made:
4.2.2 The spreading edge must be smooth (no clip out of it) and clean. Spreaders can be re-used if cleaned and dried after each use.
4.2.3 Place a small drop of blood (thoroughly but gently mixed) in the centre of the slide about 1 cm from the end. Grasp the spreader with thumb and middle finger using the index finger to put light pressure on the spreader.
4.2.4 Place the spreader beyond the drop and at an angle of 45^0 to the slide. Draw it back until it touches the drop and the blood spreads evenly across the slide. Alternatively use a complete slide as the spreader and pull it back at an angle of 450 until the blood spreads almost to the edge.

4.2.5 Quickly and smoothly push the spreader right to the end of the slide at an angle of 45^{o}.
4.2.6 Wave the slide quickly in the air to dry it. Label at the thick and with the owners name using a lead pencil.

5. Safety

- N/A

6. Trouble shoot

6.1 Once the needle is withdrawn, pressure should be maintained for 1 – 2 minutes, otherwise extravasation of blood will cause ecchymosis
6.2 Blood should immediately be transferred to appropriate containers and it should be level

7. References

- N/A

D. SKIN SCRAPINGS COLLECTION.

1. Purpose:

1.1 For confirmatory diagnosis and it is highly essential to provide efficient treatment and effective control & preventive measures.
1.2 To carry out the surveillance or prevalence of economically important diseases.

2. General information/responsibility:

The skin scrapings are mainly collected from those animals suffering from skin problems like scabies, alopecia and dermatitis in order to identify the different types of mites, nematodes and fungus that cause the skin problem.

3. Equipment/materials:

3.1 Rubber gloves
3.2 Marker pen
3.3 Glycerol,
3.4 Scissor,
3.5 Scalpel blade,
3.6 Cotton,
3.7 Spirit
3.8 Aluminium foil paper

4. Procedure:

4.1 The skin scrapings are collected from the affected parts of the skin.
4.2 The hair over the area should be clipped short and discarded. The site should be cleaned and disinfected.
4.3 The area selected should be moist part on the edge of lesions since most mites will be found on the periphery of active lesions.

4.4 For different types of mange or scabies a sharp scalpel blade should be used. Hold the blade at an acute angle and scratch until the blood oozes out freely.
4.5 In pustular form of demodecosis, mites can be demonstrated by examination of the cheesy contents of the incised pustule.
4.6 Specimens are to be sent in 10 % KOH or 10% formalin. If ecto-parasites are seen fixed them in alcohol and send to the laboratory.
4.7 All specimens should be transferred directly into a tube that can be securely stoppered. Envelopes are unsuitable containers since active mites can easily escape.

5. Safety
- N/A

6. Trouble shoot
- N/A

7. References
- N/A

8. Appendix
Preparation of preservative (10% Potassium hydroxide)

This preservative is used for preserving skin scraping or mange mites.
10 grams – Potassium hydroxide
100ml – Distilled water

E. TISSUE ASPIRATES COLLECTION

1. Purpose:
1.1 For confirmatory diagnosis and it is highly essential to provide efficient treatment and effective control & preventive measures.
1.2 To carry out the surveillance or prevalence of economically important diseases.

2. General information/responsibility:
In parasitology, tissues aspirates are mainly of lymph nodes where in the suspected cases of haemoprotozoan parasites can be ruled out. Diagnostic laboratories require the submission of appropriate samples that arrive at the laboratory in good condition. For disease diagnosis, the tissues sampled should be representative of the condition being investigated and the lesions observed.

The samples should be carefully packaged, labelled, and transported to the laboratory by the fastest practicable method, with the appropriate temperature control. There are specific requirements for the packaging and shipping of infectious substances, including diagnostic specimens that must be followed. If material is to be sent to a laboratory in another country, this laboratory should be consulted in advance to ensure that it is willing to receive the material and to obtain the appropriate import licence.

All samples should be accompanied by a letter or submission form, which includes the name and address of the submitter, the origin of the material, the relevant history, animal identification and corresponding specimens, and the tests requested.

3. Equipment/materials:

3.1 Gauge plastic needle
3.2 10 ml syringe
3.3 Glass slides

4. Procedure:

Lymph node aspirates

4.1 Wash the site of aspiration and then shave the area and clean it with povidone iodine
4.2 Now hold the palpable lymph node with the left hand tightly and fix in a position
4.3 Using a 18 G needle, puncture the lymph node and then with a syringe aspire the contents
4.4 Now the contents drawn in the syringe are spread over the glass slide and air dried. This is then send to the laboratory

5. Safety

- N/A

6. Trouble shoot

- N/A

7. References

- N/A

F MEAT SAMPLE MUSCLE BIOPSIES COLLECTION.

1. Purpose:

1.1 For confirmatory diagnosis and it is highly essential to provide efficient treatment and effective control & preventive measures.
1.2 To carry out the surveillance or prevalence of economically important diseases.
1.3 Monitor the health of the animals.

2. General information/responsibility:

In parasitological procedure meat and muscle biopsies are mainly collected to diagnose those parasites residing in the muscle. One of the nematode whose cyst resides in the muscle is the zoonotic parasite, *Trichinella spiralis.*Diagnostic laboratories require the submission of appropriate samples that arrive at the laboratory in good condition. For disease diagnosis, the tissues sampled should be representative of the condition being investigated and the lesions observed.

3. Equipment/materials:

3.1 Rubber gloves
3.2 Marker pen
3.3 Specimen container
3.4 Laboratory submission forms

4. Procedure:

4.1 In case of the dead animal which has died of suspected trichinosis, a piece of diaphragm/ heart/lungs of muscles can be collected.
4.2 In the case of whole carcasses of domestic swine a specimen weighing at least 1 g has to be taken from a pillar of the diaphragm at the transition to the sinewy part. Special trichinae forceps can be used if an accuracy of 1 g to 2.15 g can be guaranteed.
4.3 In the case of breeding sows and boars, a large sample weighing at least 2g has to be taken from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimens of twice the size 2 g for 4 g in the case of breeding sows and board) had to be taken from the rib part or from the jaw muscle, tongue or abdominal muscles
4.4 For cuts of meat, a sample weighing at least 5 g of striated muscle, containing little fat has to be taken if possible form close to bones or tendons. A sample of the same size has to be collected from meat that is not intended to be cooled thoroughly or other types of past-slaughter processing.
4.5 For frozen samples, a sample weighing at least 5 g of striated muscle tissue has to be taken for analysis. The weight of meat specimens relates to a sample of meat that is free of all fat and fascia. Special attention must be paid when collecting muscle samples from the tongue in order to avoid contamination with the superficial layer of the tongue. Which is indigestible and can prevent reading of the sediment
4.6 Muscle may be saved in saline moistened gauze for several hours.
4.7 Keep specimen cool.
4.8 Muscle should NOT be immersed in saline, fixative or other liquids.
4.9 Frozen muscle may be safely shipped "overnight" with adequate dry ice.

5. Safety

- N/A

6. Trouble shoot

- N/A

7. References

- N/A

G. GASTRO INTESTINAL TRACT.

1. Purpose:

1.1 For confirmatory diagnosis and it is highly essential to provide efficient treatment and effective control & preventive measures.

1.2 To carry out the surveillance or prevalence of economically important diseases.
1.3 Monitor the immune response of the vaccine following vaccination.
1.4 Monitor the health of the animals.
1.5 For health certification of animals for both export and import purposes

2. General information/responsibility:

Post-mortem parasite counts provide a more precise assessment of parasite burdens than parasite egg counts. For parasite counts, the intestinal tract from abomasum to rectum is required. The adult and larval nematodes are carefully washed out, counted and identified.

3. Equipment/materials:

2.1 Rubber gloves
2.2 Petri dish
2.3 Marker pen
2.4 Polythene bag
2.5 10% formalin
2.6 Spatula

4. Procedure:

4.1 In cases of suspicion during the post-mortem the total gastro-intestinal tract should be submitted to laboratory.
4.2 The tract should not be opened and each part should be tied off at the appropriate junction that, between the stomach and the small intestine between the small and large intestine.
4.3 The tract should be placed in polythene bag.

2. Safety

- N/A

3. Trouble shoot

- N/A

4. References

- N/A

5. Appendix

Preservative used for preserving nematodes (round worms)

For nematodes, put the worms into the beaker of 5% Formaldehyde solution heated to 70-82 degree Celsius. Leave this to cool and store the specimens in clear fluid of the same composition so that the morphology does not change

Preparation of preservative (10% formalin)

Formaldehyde – 5ml

Distilled water – 95ml

H. EXTERNAL PARASITES FOR IDENTIFICATION (INCLUDING INSECTS)

1. Purpose:

1.1 For confirmatory diagnosis and it is highly essential to provide efficient treatment and effective control & preventive measures.
1.2 To carry out the surveillance or prevalence of economically important diseases.
1.3 Monitor the health of the animals.

2. General information/responsibility:

Identification of ectoparasites is very important as some may also act as vectors of viruses, rickettsia, bacteria, protozoa, cestodes and nematodes, including vectors of zoonotic diseases in humans.
Identification of ticks is made possible by morphological examination of body parts like structure of mouth parts. However, accurate identification of ticks to the species, particularly of the larval and nymphal stages requires specialist knowledge and equipment.

3. Equipment/materials:

3.1 Rubber gloves
3.2 Marker pen
3.3 wide mouth screw capped bottle
3.4 Alcoholic-glycerine solution (9 parts of 80% alcohol to 1 part of glycerine**)**
3.5 Laboratory submission forms
3.6 Ticks twister

4. Procedure:

4.1 A small hairbrush dipped in ethanol may be used for the collection of ticks.
4.2 The point of attachment can be smeared with ethanol.
4.3 Adequate precautions should be taken to preserve the mouthparts and appendages of the ectoparasites during collection.
4.4 Ectoparasites should be preserved in 70% alcohol in clean, well-stopper glass vials which should be labelled properly or alcoholic-glycerine solution (9 parts of 80% alcohol to 1 part of glycerine) in a small leak-proof container.

5. Safety

- N/A

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6. **Trouble shoot**

- N/A

7. References

http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e05.htm accessed at 4 PM on 31/08/2018
https://www.rvc.ac.uk/review/parasitology/Faeces/Step1a. htm accessed at 4 PM on 31/08/2018
https://www.merckvetmanual.com/clinical-pathology-and-procedures/collection-and-submission-of-laboratory-samples/overview-of-collection-and-submission-of-laboratory-samples 4 PM on 31/08/2018

TITLE: Faecal examination by Direct Method

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
It is possible to demonstrate the presence of the eggs of Nematodes, Cestodes, Trematodes and the oocysts of *Coccidia* by the examination of a thin smear of emulsified faecal material. The demonstration of eggs or larvae in faeces can indicate the presence of parasitic infection and facilitate the diagnosis of parasitic disease.

2. PRINCIPLES
Eggs and larvae produced by adult helminths in the host animal are often passed out in the faeces.

3. APPLICATION
This test used to demonstrate the presence of eggs of helminths or protozoa by the examination of a thin smear of emulsified faeces

4. OBJECTIVE
To describe the process to demonstrate the presence of eggs of helminths or protozoa by the examination of a thin smear of emulsified faeces

5. APPARATUS
5.1 Glass slides (preferably 25x 75mm)
5.2 Cover glass (preferable 22x40)
5.3 Tooth pick or applicator
5.4 Microscope

6. REAGENTS, SOLUTION AND BUFFER
6.1 Iodine solution
6.2 Saline or tape water

7. PROCEDURE
7.1 Place 2 to 3 drops of water or saline in the centre of a glass slide.
7.2 Using an applicator, take a small quantity of the faeces and mix with water or saline. The slummy should be thin enough to read type print through. The largest particles can be moved aside.

7.3 Apply 22x40 mm cover glass and examine the entire area with low power objective, changing to high when necessary for parasitic ova, cysts and larvae. Examination will be difficult if too much water or too much faeces are used.

7.4 The sample can also be prepared in a few drops of physiological saline solution or in a few drops of iodine solution. With this method the egg shell e.g. S*chistosoma* or *Protozoa* e.g. *Giardia lamblia* are stained.

8. RESULT INTERPRETATION

- Parasitic ova will be identified as per their morphological characters.

9. WASTE DISPOSAL

- Appropriate hygiene and safety procedures should be employed.

10. RISK ASSESSMENT

- Outmost care should be taken to avoid the infection to the collector. If the faeces contain an infectious organisms like coccidia, giardia and Toxoplasma etc. Can be infectious to people.

11. TROUBLESHOOTING

- It can be difficult to observe or identify the eggs as they may be partly or completely covered by debris.

12. REFERENCES

- RVC/FAO guide to Veterinary Diagnostic Parasitology

TITLE: Faecal examination by Direct Method – Mucosal Impression Smear

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
This includes the recovery of parasites whose site of predilection or the parasites or their ova are found along the mucosal lining of intestine. This is also a post mortem indication for recovery of parasitic infestation. All the procedure for examination of parasites remains the same.

2. PRINCIPLES
This test is a simple technique for the detection of coccidia oocyst during post-mortem examination.

3. APPLICATION
This test is used for the detection of coccidia oocyst during post-mortem examination using faecal examination by Direct Method – Mucosal Impression Smear Examination

4. OBJECTIVE
To describe the procedure for the detection of coccidia oocyst during post-mortem examination using faecal examination by Direct Method – Mucosal Impression Smear Examination

5. APPARATUS
5.1 Glass slides
5.2 Cover slips
5.3 Paper towel
5.4 Microscope

6. REAGENTS, SOLUTION AND BUFFER
- Giemsa's stain

7. PROCEDURE
7.1 Gently blot the mucosal surface with a paper towel to remove excess fluid and debris.
7.2 Press a microscope slide firmly against the mucosa using a slight rotating movement or use mucosal scrapings.
7.3 Lift the slide directly away from the mucosa, air dry the smear, stain with Giemsa stain and examination under the microscope.

If scrapping is used, put cover slip and directly see under the microscope.

8. RESULT INTERPRETATION

Different morphological characters of Coccidian oocyst are shown in annexure.
Aided by the post-mortem finding and part of mucosa examined, the kind of infection can be identified though in case of coccidian, the morphological characters are not distinct between the types

9. WASTE DISPOSAL

Appropriate hygiene and safety procedures should be employed.

10. RISK ASSESSMENT

Outmost care should be taken to avoid the infection to the collector. If the faeces contain an infectious organisms like coccidia, *Giardia* and *Toxoplasma* etc. Can be infectious to people.

11. TROUBLESHOOTING

It is mostly not applicable in live animals to obtain the mucosal smear from the different parts of intestine.

12. REFERENCES

Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food (MAFF/ ADAS) London, Her Majesty's Stationary Office (1971) Reference Book 418.

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

TITLE: Flotation Technique

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

A number of different methods are available for separating, concentrating and demonstrating eggs, oocysts and larvae in faecal samples. Three methods are described for ready use for qualitative estimation of parasitic infestations

1.1 Simple test tube flotation
1.2 Simple flotation
1.3 Sedimentation technique (for Trematode eggs)

All flotation technique involves the advantage of a difference in the Buoyancy of parasites relative to food residues. When some faeces are suspended in water, the eggs and solid faecal particles will settle down, allowing the supernatants and dissolved pigments to be decanted. Due to difference in the density of eggs of different parasites, the same principle works to differentiate the parasitic ova by layers.
Simple Flotation Method is another good technique for use in initial surveys. In addition, it can be used in Conjunction with the McMaster technique to detect low numbers of eggs (when Present below the Mc Master sensitivity of 50 eggs per gram of faeces).

2. PRINCIPLES

Faeces are mixed with a floatation solutions which has a higher specific gravity than water to give suspension. Heavy faecal particles sink or remain in suspension, while specifically lighter parasite stages ascend and accumulate on surface.

3. APPLICATION

This test is used for the detection of nematode and cestode eggs and coccidian oocysts in the faeces using Flotation Method

4. OBJECTIVE

To describe the procedure to detect nematode and cestode eggs and coccidian oocysts in the faeces.

5. APPARATUS

5.1 Beakers or plastic containers
5.2 A tea strainer (preferably nylon) or double layer cheese cloth
5.3 Measuring cylinder or other container graded by volume
5.4 Fork, tongue blades or other type of stirring rod

5.5 Test tube
5.6 Test tube rack or a stand
5.7 Micro slides, cover slips
5.8 Balance or teaspoon
5.9 Microscope

6. REAGENTS, SOLUTION AND BUFFER

- Floatation fluid
  - ✓ Saturated NaCl sG= 1.2g
  - ✓ Saturated Sugar solution sg=1.3g
  - ✓ $ZnCl_2$=sG=1.3g

7. PROCEDURE

7.1 Simple Test Tube Floatation Method

7.1.1 Put approximately 3 g of faeces (weigh or measure with a pre calibrated tea spoon) into
7.1.2 Pour 50 ml flotation fluid into Container 1.
7.1.3 Mix (stir) faeces and flotation fluid thoroughly with a stirring device (tongue blade, fork)
7.1.4 Pour the resulting faecal suspension through a tea strainer or a double layer of cheese cloth into Container 2.
7.1.5 Pour the faecal suspension into a test tube from Container 2.
7.1.6 Place the test tube in a test tube rack or stand.
7.1.7 Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a cover slip on top of the test tube. Let the test tube stand for 20 minutes.
7.1.8 Carefully lift off the cover slip from the tube, together with the drop of fluid adhering to it, and immediately place the cover slip on a microscope slide.

7.2 Simple Floatation method

7.2.1 Put approximately 3 g of faeces (weigh or measure the faeces with a pre-calibrated teaspoon) into container 1
7.2.2 Pour 50 ml of flotation fluid into container 1.
7.2.3 Mix (stir) the contents thoroughly with a stirring device (tongue blade, fork).
7.2.4 Poured the resultant faecal suspension through a tea strainer or a double layer of cheesecloth into container 2.
7.2.5 Leave the container to stand for 10 minutes.
7.2.6 Press a test tube to the bottom of the filtrate, lift it quickly and transfer a few drops adhering to the surface to a micro slide.
7.2.7 The test tube ought to touch the micro slide for at least 2-4 seconds for the drops to run off.
7.2.8 Mount the cover slip on the micro slide for microscopically examination.

8. RESULT INTERPRETATION

Nematode and cestode eggs will float and the trematode eggs will sink, which can be further classified through their morphological characters. The quantitative value shall be described in EPG

9. WASTE DISPOSAL

- Appropriate hygiene and safety procedures should be employed.

10. RISK ASSESSMENT

- Outmost care should be taken to avoid the infection to the collector. If the faeces contain an infectious organisms like Coccidia, giardia and Toxoplasma etc. Can be infectious to people.

11. TROUBLESHOOTING

- In general, techniques based on the flotation principle works well for nematode and cestode eggs and protozoon cyst but fails to float some trematode eggs. Therefore, sedimentation techniques is used for trematodes eggs

12. REFERENCES

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

TITLE: Sedimentation technique

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
The sedimentation technique is qualitative method for detecting trematode eggs (*Paramphistomum*) in the faeces. Most trematode eggs are relatively large and heavy compared to nematode eggs. This technique concentrates them in sediment.

2. PRINCIPLES
In a faeces-water suspension, parasite eggs with relatively high specific gravity (e.g. trematode eggs) and heavy faecal particles sink rather quickly to the bottom and concentrate in the sediment. By repeated sedimentation and decantation of the supernatant, the lighter faecal particles are removed and the parasite stages cane be detected in the final sediment.

3. APPLICATION
- This test is used for detecting trematode eggs (*Paramphistomum*) in the faeces

4. OBJECTIVE
- To describe the procedure for detecting the trematodes eggs through sedimentation techniques

5. APPARATUS
5.1 Beakers or plastic containers
5.2 A tea strainer or cheese cloth
5.3 Measuring cylinder
5.4 Stirring device (fork, tongue blade)
5.5 Test tubes
5.6 Test tube rack
5.7 Micro slide, cover slips
5.8 Balance or teaspoon
5.9 Microscope

6. REAGENTS, SOLUTION AND BUFFER
- Methylene blue

7. PROCEDURE
7.1 Weigh or measure approximately 3-10g of faeces into Container 1.
7.2 Pour 45 ml of tap water into Container 1.

7.3 Mix (stir) thoroughly with a stirring device (fork, tongue blade)
7.4 Filter the faecal suspension through a tea strainer or double-layer of cheesecloth into Container 2.
7.5 Pour the filtered material into a test tube.
7.6 Allow to sediment for 5 minutes.
7.7 Remove (pipette, decant) the supernatant very carefully.
7.8 Re suspend the sediment in 5 ml of water.
7.9 Allow to sediment for 5 minutes.
7.10 Discard (pipette, decant) the supernatant very carefully.
7.11 Stain the sediment by adding one drop of methylene blue.
7.12 Transfer the sediment to a micro slide Cover with a cover slip.

8. RESULT INTERPRETATION

- See annexure for the morphology of trematode eggs.

9. WASTE DISPOSAL

- Appropriate hygiene and safety procedures should be employed.

10. RISK ASSESSMENT

- Outmost care should be taken to avoid the infection to the collector. If the faeces contain an infectious organisms like Coccidia, giardia and Toxoplasma etc. Can be infectious to people.

11. TROUBLESHOOTING

- N/A

12. REFERENCES

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

TITLE: Quantitative Techniques – Stoll's Dilution Method

PREAPERED: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU.

DATE: 11.06.2018

1. INTRODUCTION
The simplest and most effective method for determining the number of eggs or oocysts per gram of faeces is the Quantitative techniques for separating and concentrating**.**

2. PRINCIPLES
This is a simple dilution procedure which facilitates the recognition of eggs and permits a quantitative determination of their concentration in the faeces.

3. APPLICATION
- This test is used for determining the number of eggs or oocysts per gram of faeces of animal

4. OBJECTIVE
- To describe the procedure for determining the number of eggs or oocysts per gram of faeces of animal

5. APPARATUS
5.1 Beaker/ Plastic container
5.2 Balance
5.3 Tea strainer or Cheese cloth.
5.4 Measuring cylinder
5.5 Stirring device(fork, tongue depressor)
5.6 Pasteur pipettes and rubber teats
5.7 Microscopic
5.8 Glass slide/ Cover slip
5.9 Glass balls.

6. REAGENTS, SOLUTION AND BUFFER
NA

7. PROCEDURE
7.1 Approximately 45 glass balls are put into a shaker jar with 45 ml of water.
7.2 Put 3 grams of faecal in the jar.
7.3 The stopper is fitted to the jar, which is shaken until all the faecal matter is broken down.

7.4 The mixture is poured through a wire mesh screen with an aperture of 0.15 mm and the strained fluid caught in a bowl. The debris left on the screen is discarded.
7.5 The filtrate of faeces is well stirred and 0.15 ml of the fluid is taken out by means of a graduated pipette.
7.6 The measured quantity of fluid is ejected on to a microscope slide and covered with a 22x22 mm cover glass and examined under microscope.

8. RESULT INTERPRETATION

The total number of eggs present in the 0.15 ml of diluted faeces is multiplied by 100 to give the number of eggs per gram of the original faecal sample.

9. WASTE DISPOSAL

- Appropriate hygiene and safety procedures should be employed.

10. RISK ASSESSMENT

- Outmost care should be taken to avoid the infection to the collector. If the faeces contain an infectious organisms like Coccidia, giardia and Toxoplasma etc. Can be infectious to people.

11. TROUBLESHOOTING

- N/A

12. REFERENCES

Manual of veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office London, UK

TITLE: Faecal culture examination for infective larvae

PERPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

Many nematode eggs are alike and species such as *Heemonchus, Mecistocirrus, Ostertagia, Trichstrongylus, Cooperia, Banostomum, and Oesophagostomum* cannot be clearly differentiated from the eggs in faecal samples. For these parasites, differentiation can be achieved by the use of faecal cultures.

Culture bottle method is a technique used in the culture of parasites into infective larvae for identification. A large sample of faeces must be cultured because there are relatively fewer worm eggs in faeces of cattle. Because of this, the culture bottle method is used for culturing cattle faeces and recovery of infective nematode larvae for identification.

2. PRINCIPLES

The muscle larvae are released after digestion of muscle tissues by means of artificial digestion using fluid composed of pepsin and hydrochloric acid. The digestion is then followed by selective screening, filtration or sedimentation procedures and a final microscopic examination for the presence of larvae.

3. APPLICATION

- This test is used for faecal culture examination for infective larvae

4. OBJECTIVE

- To describe the procedure for faecal culture examination for infective larvae

5. APPARATUS

5.1 Faecal culture examination by culture jars method

5.1.1 Fork
5.1.2 Spoon
5.1.3 Tongue depressor
5.1.4 Spatula
5.1.5 Water Jars and containers
5.1.6 Charcoal dried
5.1.7 Sterile bovine faeces may be used if charcoal is not available. This is prepared by sterilizing the faeces to remove any Helminth eggs present and then completely dried by heating to 70^0C and ground to a fine powder)

5.2 Culture of larvae from faeces of cattle using culture bottles

5.2.1 Culture bottles, extra-wide-mouthed, 4 fl. oz. capacity as used for collecting samples of faeces from cattle. Lids are required.
5.2.2 Charcoal or sterilized bovine faeces.
5.2.3 Petri dishes
5.2.4 Pasteur pipettes.
5.2.5 Glass slides or larval chamber slide.
5.2.6 Microscope.

6. REAGENTS, SOLUTION AND BUFFER

- NA

7. PROCEDURE

7.1 Faecal culture examination by culture jars method

7.1.1 Break up collected faeces finely using a stirring devices
7.1.2 Faeces should be moist and crumbly. If faeces are too dry, add water. If faeces are too wet, add charcoal (or sterile bovine faeces) until the correct consistency is obtained.
7.1.3 Transfer the mixture to jars or other container.
7.1.4 Leave the culture at room temperature for 14 to 21 days, by which time all larvae should have reached the infective stage.
7.1.5 If an incubator is available, the culture can be placed at 27 ^{0}C and kept for 7 to 10 days.
7.1.6 Add water to cultures regularly (every 1-2 days)
7.1.7 Larvae are recovered using the Baermann techniques (Refer SOP for Baermann's Technique).

7.2 Culture of larvae from faeces of cattle using culture bottles

7.2.1 Take 18-20 g faeces and mix and adjust for consistency and moisture content by adding charcoal or sterilized bovine faeces.
7.2.2 Place in culture bottles with the lids screwed on lightly and incubate for seven days at 25 ^{0}C – 27 ^{0}C.
7.2.3 After incubation, remove lids and completely fill the bottle with water, place a Petri dish over the top of the bottle, hold it there firmly and invert the two together.
7.2.4 Place the Petri dish on a slightly inclined plane with the culture bottle standing in it, upside down.
7.2.5 Add water to the Petri dish until the water level surrounds the mouth of the bottle.
7.2.6 Allow to stand for half to 1 hour. The larvae will move from the sample to the water in the Petri dish, collecting at the lowest position from whence they can be taken off with a Pasteur pipette for counting.

8. RESULT INTERPRETATION

The egg of the nematode worms of ruminants hatches into a first stage larva. This develops into the second stage larva. Larvae in these first two stages are not infective.

When the third stage larva develops it retains around itself the skin or sheath of the second stage larva. This is the infective stage that develops on through a fourth stage to the adult worms on entering the host animal.
It is the third stage infective larva that is recovered from cultures after 7 days incubation. The sheath of the second stage larva that surrounds it is very important in differentiation of species. The infective larvae are identified chiefly by comparative overall length, from end of tail of larva to end of sheath and shape of the tail of the larva and of the sheath. Other identification points, such as head characteristics, numbers of intestinal cells etc, are also used. Proficiency in identification comes from constant practice.

Total length of larva (u)	***Length, end of larvae to end of sheath (u)***	***Species, with range of total length (u)***	***Key to opposite page***	***Other differential features***
Short 500-700	No sheath	*Strongyloides* 570-700	A	Slender body with long oesohagus.1/3 to ½ total length of larvae.
	Long 85-115	*Bunostomum* 510-790	B	Wide body with sudden tapering to long thin tail. "Band" constriction on oesophagus.
Medium 650-900	Short 20-40	*Trichostrongyliasis* 620-790	C	Short straight larvae, conical tail sheath. Tubercles on tail of larva. Intestinal cells usually prominent.
		Ostertagia 790-910	D	Longer, conical, "finger-like" tail sheath.
Medium 650-900		*Cooperia curtises* 710-910	E	Oval bodies at anterior end of larva. Tail of larva rounded.
	Medium 30-60	*Haemonchus* 650-750	F	Tail sheath is usually "kinked" pointed tail of larva.
		Cooperies oncophera 800-920	G	

9. WASTE DISPOSAL

- NA

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

11.1 If the egg count is low, it does not yield the sufficient larvae. In this case, faecal mass can be transferred to a Baermann apparatus, as is used for lungworm larval recovery and a higher field will usually be obtained.

11.2 Note that in examining larvae cultured from faeces it is common to find types not typical of any of the infective nematode larvae. Free living forms of *Strongyloides* are frequently seen and these include several types, but usually they are readily distinguishable by a rhabditiform oesophagus. Some cross infection occurs between sheep and cattle, so the cattle species of *Haemonchus*, which is larger than the sheep species, may sometimes be seen in sheep samples. The infective nematode larvae may sometimes shed the sheath and present confusing forms. Also, first or second stage larvae may be seen.

12. REFERENCES

Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food, HMSO London, UK. Reference book: 418.

TITLE: Baermann technique for detecting nematode larvae

PERPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
The culture of lungworms from faeces and the recovery of infective larvae can be used as an aid for clinical diagnosis of lung worm infection as well for the general investigation. This technique is probably the most widely used method for the recovery of nematode larva from faeces and faecal culture.

2. PRINCIPLES
The living nematode larvae move out from faeces (soil, animal tissues) into surrounding water and are concentrated in a funnel.

3. APPLICATION
- This procedure is used for Isolation and identification of nematode larvae and infective larvae harvested from faecal cultures.

4. OBJECTIVE
- To describe the process of Isolation and Identification of lungworm and infective larvae using Baermanns technique.

5. APPARATUS
5.1 Funnel (size according to need) and funnel stand.
5.2 Rubber or plastic tubing and rubber bands
5.3 Clamp or spring clip and cheese cloth or screen
5.4 Simple thin stick (about 15 cm long)
5.5 Strainer, test tube and Pasteur Pipettes.
5.6 Small Petri dishes and Microscope

6. REAGENTS, SOLUTION AND BUFFER
- Iodine

7. PROCEDURE

7.1 Examination of lung worm larvae.

7.1.1 Fit a short piece of tubing which is closed at one end with a clamp or spring clip to the stem of funnel of appropriate size.
7.1.2 Support the funnel by a stand.

7.1.3 Weigh or measure about 5 to 10g of faecal culture/faeces and place it on a piece of double-layer cheese cloth.
7.1.4 Form the cheese cloth around as or pouch.
7.1.5 Close the pouch with a rubber band.
7.1.6 Fix a supporting stick under the rubber band. Place the pouch containing faecal culture material or faeces in the funnel with Luke warm water, covering the faecal material.
7.1.7 Leave the apparatus in place for 24 hours, during which time larvae actively move out of faeces and ultimately collect by gravitation in the stem of funnel.
7.1.8 Draw a few ml. of fluid from the stem of the funnel into a small Petri dish.
7.1.9 For positive samples a transfer of larvae to a micro slide for identification at higher magnifications may be required.
7.1.10 It is important to differentiation between Muellerius capillaries and other species as the treatment is difficult.

7.2 Examination for infective larvae from faecal cultures.

7.2.1 Draw 10 to 15ml of fluid from the stem of the funnel into a test tube or other container.
7.2.2 Leave the tube to stand for 30 minutes. Remove the supernatant with a Pasteur pipette.
7.2.3 Transfer a small aliquot of the remaining fluid using a Pasteur pipette to a micro slide, add a drop of iodine and cover with a cover slip.
7.2.4 Examination under 10x 10 magnifications for larval identification.
7.2.5 Repeat steps 1 and 2 until 100 larvae had been identified.
7.2.6 The counts for each species provide an estimate of the composition (%) of the parasite population of the host.

7 RESULT INTERPRETATION

In the case of lungworm infected animals, the larva excretion may be expressed as larva per gram of feces or compared with the theoretical number of eggs present in the original culture to give relative indication of the proportion of viable eggs present.

Key for the identification of the third-stage larvae of some common gastro-intestinal nematodes of sheep and cattle

1.	*Oesophagus rhabditiform*	Free-Living nematode
	Oesophagus not rhabditiform	2
2.	Without sheath, oesophagus nearly half the length of the body with Sheath,	*Strongyloides*
	With Sheath, oesophagus less than the quarter the length of the body	3
3.	Tail of sheath short, non-filamentous.	4
	Tail of sheath medium length, non-filamentous.	5
	Tail of sheath filamentous.	6
4.	Head of larva tapered, tail indistinctly rounded or	*Trichostrongylus*

	bearing one or two tuberosities.	
	Head of larva squared, tail indistinctly rounded	*Ovine Ostertagia*
5.	Head of larva square, tail of sheath resembles a Cone.	*Bovine Ostertagia*
	Head of larva square, bearing refractile bodies or band.	*Cooperia*
	Tail of sheath tapering almost to a filament or abruptly becoming a fine point.	*Cooperia*
	Head of larva narrow rounded tail of sheath offset.	*Haemonchus*
6.	Head broad rounded, 8 gut cells. Larval tail, notched, bilobed or trilobed	*Nematodirus*
	Head broad rounded, 32 gut cells	*Oesophagostomum/Chabertia*
	Very small larva, 16 gut cells	Bunostomum

Key characteristics used in the identification of third-stage larvae of sheep and cattle

Genus	**Intestinal cell number**	**Head Characteristics**	**Sheath tail characteristics**
Nematodirus	8	Broad rounded	Filamentous
Ostertagia (ovine)	16	Squared	Short cone
Ostertagia (bovine)	16	Squared	Medium cone
Cooperia	16	Squared with refractile bodies	Medium tapering or finely pointed
Haemonchus	16	Norrow rounded	Medium offset
Trichostrongylus	16	Tapered	Short cone
Bunostomum	16		Short filamentous
Oesophagostomum/ Chabertia	32	Broad rounded	Filamentous

Notes:

- It must be remembered that part from the actual number of intestinal cells present in the different species and the refractile bodies of *Cooperia spp*. the other characteristics are relative rather than definitive.
- *Strongyloides* larvae have no sheath, a long oesophagus and bipartite tail.
- Porcine *Oesophagostomum spp*. has 16 intestinal cells.
- Species of *Nematodirus* are differentiated by the shape of larval tail; filicollis tends to take up iodine quite readily.
- *Haemonchus contortus* have ill-defined cells, take up iodine quite readily and genital primordium is usually readily observed.
- *Bunostomum* larvae have ill-defined cells and are the smallest larvae encountered.
- The tail of the sheath of the 'small' *Cooperia spp*. ends in a needle sharp point usually refractile in appearance as opposed to the tapering 'tap-root like' appearance of *C. oncophora*.
- A rule of thumb differentiation between ovine *Ostertagia* and *Trichostrongylus* spp is that ovine *Ostertagia* are usually >½ diameter of the field of view with 6x eyepiece

and 10x objective (1.25x tube factor). The tapered head of *Trichostrongylus* is however, diagnostic.

8 WASTE DISPOSAL

Appropriate hygiene and safety procedures should be employed.

9 RISK ASSESSMENT

- Faeces may contain hazardous pathogens (bacteria, viruses etc). Appropriate hygiene and safety procedures should be employed.

10 TROUBLESHOOTING

10.1 The reliability of detecting larvae of *Dictyocaulus viviparus* decreases significantly at temperature above 25^{o}C.

10.2 Faecal examination technique - Larva from embryonated eggs may only be detected if the faecal sample is left in the Baermann apparatus overnight allowing time for eggs to hatch and the emerging larvae to escape from the faecal sample.

11 REFERENCES

Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food, HMSO London, UK. Reference book: 418.

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

TITLE: Culture and Sporulation of Coccidian Oocyst

PERPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
Coccidiosis commonly occurs in all birds and animals, mostly infecting the lining of alimentary tract at various locations. Coccidian are strictly host-specific but their pathogenicity varies with species and location of infection

2. PRINCIPLES
The main aim of the test is to distinguish between different genera and species of coccidian parasites by the characteristics of the sporulating oocyst. This is achieved by culturing the infected faeces in cultural media for an appropriate period of time.

3. APPLICATION
- This procedure is used for the culture and sporulation of Coccidian Oocyst

4. OBJECTIVE
- To describe the procedure for the culture and sporulation of Coccidian Oocyst

5. APPARATUS
5.1 Petri dish and
5.2 Glass rod, microscopic slide and cover glass
5.3 Centrifuge
5.4 Microscope

6. REAGENTS, SOLUTION AND BUFFER
6.1 Sheather's sugar solution
6.2 Potassium dichromate solution (2.5%)

7. PROCEDURE
7.1 Mix approximately 1g of faecal material with 2.5% potassium dichromate solution.
7.2 Place the mixture in a Petri dish.
7.3 The potassium dichromate should form a 5mm layer over the faecal material when it sediments.
7.4 Let it stand at room temperature for 5 to 10 days. The length of time will depend on the species present and temperature.
7.5 Concentrate the oocyst by centrifuging contents of the petri dish at 1500 rpm for 3 to 5 minutes.

7.6 Decant the supernatant.
7.7 Add sheathers sugar solution to the sediment and mix well.
7.8 Centrifuge at 1500 rmp for 3 minutes.
7.9 Applying a glass rod to the surface film.
7.10 Transfer the drop on the rod to a microscope slide.
7.11 Apply a cover slip and examine under low magnification.

8. RESULT INTERPRETATION

Sporulation is usually complete after 2 to 4 days of incubation at room temperature**.** The key to identification of some of the important coccidian OOCYST is given in the table below:

Key to identification of chicken Coccidiosis:

Species	**Site Of infection**	**Average oocysts dimensions (microns)**	**Prepatent Period(days)**	**Pathogenicity In moderate infections**
Eimeria acervulina	Duodenum	18x14	4	Weight loss caused by Gametocytes and schizonts
E. brunetti	Lower small Intestine	26 x 22	6	Variable mortality. Weight loss. Gametocytes and schizonts present variable mortality.
E. maxima	Mid-small Intestine	30x20	6	Variable mortality. Weight loss. Mainly gametocytes present
E. mitis	Lower small intestine and proximal caeca	16x15	4	Weight loss caused by Gametocytes.
E. necatrix	Schizonts in Small intestine. Gametocytes and oocysts in Caeca	20x17	7	Schizonts deep in intestinal tissue cause haemorrhage, Weight loss and mortality.
E. praecox	Duodenum	21x17	4	Temporary Weight loss in heavy infection.
E. tenella	Caeca	23x19	7	Schizonts deep in caecal tissue cause haemorrhage and mortality.

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

11.1 Sporulating oocyst need a lot of air, so the pool must be shallow to favour the diffusion of oxygen

11.2 Do not let the culture to dry, and add more dichromate if necessary.

12. REFERENCES

Knoll, J.S. (2010). Recipe of Shaether's sucrose solution. Veterinary medicine. Extracted from website: Veterinary medicine.DVM360.com/recipe. sheather's-sucrose-solution on 10/02/2018, 11.56am

13. APPENDIX

Preparation of sheather's sugar solution –

13.1 Requirement-454gms granulated sugar, 355ml tap water, 6 ml (37%) formalin

13.2 Heat the tap water to near boiling.

13.3 Add granulated sugar and stir until the sugar is dissolved.

13.4 Allow the mixture to cool to room temperature and add the formalin.

13.5 Check the solution specific gravity and adjust it to 1.27 by adding water or sugar.

TITLE: Modified Sedimentation Technique for the detection of *Fasciola* eggs.

PERPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

The ST detects with sufficient reliability oocysts of *Eimerialeuckarti*, and eggs of *Fasciola*, *paramphistomes* or *schistosomes*. The analytical sensitivity of the ST for detecting *Fasciola* eggs is very high (close to 100%) if epg (egg counts per G of faeces) are between 10 and 1000; the lower limit of detection is 3-4 epg. In examinations of animals naturally infected with *F. hepatica*, not all parasite carriers are detected, as egg shedding is highly variable and often low, especially in cattle. Thus, diagnostic sensitivities in cattle of 60-70% can be expected, whereas in sheep>80%. Repeated examinations of each 10 g of faeces of the same batch revealed an average sensitivity of 69% the examination of 3 samples increased the sensitivity to 90% (using 20 g we can expect a sensitivity of 86%). The specificity is high because the most related eggs of *Paramphistomum*spp. Can be differentiated by colour and size.

The following quantities of faecal samples are used for this method: cattle and horse 20 g, small ruminants 10 g. Hard and dry faeces of small ruminants can be soaked in water for 1 hr.

2. PRINCIPLES

In a faeces-water suspension, parasite eggs with a relatively high specific gravity (e.g. trematode eggs) and heavy faecal particles sink rather quickly to the bottom and concentrate in the sediment. By repeated sedimentation and decantation of the supernatant, the lighter faecal particles are removed, and the parasite stages can be detected in the final sediment.

3. APPLLICATION

This is a procedure to assess the presence of trematode infections. It is generally run only when such infections are suspected (from previous post-mortem findings on other animals in the herd/flock area), but it can be run routinely. The procedure can be used to detect liver fluke (*Fasciola*) and *Paramphistomum* eggs.

4. OBJECTIVE

- To describe the procedure for detection of *Fasciola* eggs using Modified Sedimentation Technique.

5. APPARATUS

5.1 Beakers or plastic containers

5.2 A tea strainer or cheese cloth

5.3 Measuring cylinder
5.4 Stirring device (fork, tongue blade)
5.5 Test tubes
5.6 Test tube rack
5.7 Micro slide, cover slips
5.8 Balance or teaspoon
5.9 Microscope

6. REAGENTS, SOLUTION AND BUFFER

- Methylene blue

7. PROCEDURE

7.1 Mix 20 g faeces with around 50-100 ml water to make a homogeneous suspension

7.2 Pour suspension through a fine-meshed sieve (mesh size 100-300 µm) into a 500 ml beaker

7.3 Wash the sieving residue with strong water jet and fill beaker up to 350 ml

7.4 Let the suspension sediment for only 5 min, decant the supernatant, fill the beaker with water up to 250 ml and repeat the procedure once (a) or twice (b).

a) Once for the investigation by microscope, in this case after the second wash step give the sediment in a 15 ml tube and again sediment it for 10 min, then carful decant the supernatant. This sediment can be investigated on a slide.

b)Twice for the investigation by Binocular: in this case add a few drops of methylene blue (1%solution) to the sediment, transfer the sediment to a Petri dish with counting grid and examine (one -3 Petri dishes have to be used depending on the purity of the sample)

8. RESULT INTERPRETATION

All Sediment has to be investigated in both a) or b) in negative cases or in cases with few eggs (under 20 eggs). In cases with many eggs not all the sample has to be investigated. (For exact quantitative analyses see McMaster-Method or please count all eggs and calculate egg/g (total number of eggs divided with 20).

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

11.1 In case of a time delay between processing the sample and reading the count, egg number may decline dramatically. Also, eggs may change their appearance, becoming crenated and “ghost-like”. It is therefore advisable to prepare only a few

samples at the time. These changes can be prevented by keeping prepared samples in the refrigerator after mixing.

11.2 In examinations for *schistosome* eggs, physiological saline solution has to be used instead of water (miracidia hatch in water, empty eggs do not sediment).

12. REFERENCES

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

TITLE: Floatation technique for *Taeniid* eggs

PERPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.218

1. INTRODUCTION

Cysticercosis in various farm and wild animals and in humans are caused by metacestodes (or larval cestodes) of *Taenia* spp. Tapeworms. Adult tapeworms are found in the small intestine of carnivore definitive hosts –humans, dogs, and wild canids. *Taenia saginata* of humans causes bovine cysticercosis, which occurs virtually world-wide. *Taenia solium* of humans causes porcine cysticercosis and human neurocysticercosis (NCC).
The eggs of *Taenia* spp and *Echinococcus* spp cannot be differentiated by microscopic examination;

2. PRINCIPLES

The flotation method is a qualitative test for the detection of *Taenia* eggs in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a floatation fluid with an appropriate specific gravity.

3. APPLLICATION

- This test is used for the detection of *Taenia* eggs in the faeces

4. OBJECTIVE

- To describe the procedure for detection of *Taenia* eggs in the faeces by floatation techniques

5. APPARATUS

5.1 Beakers or plastic containers
5.2 A tea strainer or cheese cloth
5.3 Measuring cylinder
5.4 Stirring device (fork, tongue blade)
5.5 Test tubes
5.6 Test tube rack
5.7 Micro slide, cover slips
5.8 Balance or teaspoon
5.9 Microscope

6. REAGENTS, SOLUTION AND BUFFER

- Sugar solution

7. PROCEDURE

7.1 About 3 g of faeces are taken in a 50 ml tube and added the 45ml of (1:2) concentrated sugar solution.

7.2 After thoroughly shaking, the mixture is centrifuged at 1500 to 2000 RPM for 10 minutes.

7.3 The supernatant containing taeniid eggs (diameter about 32 µm) is sieved with mesh sizes 100 µm and then 41 µm, and then sieved with a mesh of 20µm, where eggs are retained.

7.4 Both the 100 µm and then 41 µm sieves are washed with water, and then the eggs are sucked by a Pasteur pipette from the 20 µm sieve.

7.5 Collected material is examined microscopically for taeniid eggs. If eggs were detected, the material is centrifuged 15000 RPM for 2 minutes and the pellet will be stored at -80^{0}C for DNA extraction

8. RESULT INTERPRETATION

All the *taeniid* eggs will appear circular with radially striated egg shell.

9. WASTE DISPOSAL

9.1 The faecal samples should at least stored at $^-$70 to $^-$80^{0}C for minimum 3 days .By this the infectivity of the eggs are lost then only they can be processed for examination. Same with the hydatid cysts, after the study or examination, should be dipped in dipped in 2% sodium hypochlorite solution for about 1 hr. And then can be disposed off.

9.2 The materials after the tests should be dipped in 2% sodium hypochlorite solution for about 1hr. Then it should be washed thoroughly and then only be disposed or reused in case of glassware.

10. RISK ASSESSMENT

- There is a risk of infecting human from the taeniid eggs. Hence, proper measures should be taken while handling the samples.

11. TROUBLESHOOTING

The eggs of *Taenia* spp and *Echinococcus* spp cannot be differentiated by microscopic examination; PCR methods are required. Direct microscopic examination of fecal samples or fecal flotation may reveal the eggs of *Spirometra mansonoides*, which are sometimes mistaken for trematode eggs, although they are larger and possess an operculum that is often difficult to see.

12. REFERENCES

Mathis, A., Deplezes, P., Eckert, J. (1996). An improved test system for PCR based specific detetction of *Echinococcous multilocularis* eggs. J. Helminthol.70. 219-222.

Bowman DD. Diagnostic parasitology. In: Bowman DD, editor. Georgi's parasitology for veterinarians. 9th ed. St-Louis: Elsevier; 2009. p. 295–371.

TITLE: Post-mortem recovery and differential parasite count

PERPARED BY: Parasitology section

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DATE: 11.06.2018

1. INTRODUCTION

Post-mortem parasite counts provide a more precise assessment of parasite burdens than parasite egg counts. For parasite counts, the intestinal tract from abomasums to rectum is required. The adult and larval nematodes are carefully washed out, counted and identified. In addition, a complete post-mortem examination of all organs should be alone, bearing in mind alternative cases of ill health or death. It is important to record all abnormalities and lessons observed. A number of parasites will be found in almost every grazing animal, irrespective of the state of its health. To assess the significance of parasite infections in field mortalities, it is therefore necessary not only to determine the species present, but also to assess the number of each species.

Methods suitable for differential parasite counting under field or laboratory conditions using simple, easily obtainable and inexpensive equipments are described in the following headings. Counts of gastro-intestinal parasites are most conveniently done by examining the abomasums, small intestine and large intestines separately. The following techniques are quantitative procedures for isolating, counting and identifying adult and larval nematodes in the abomasums and adult nematodes in the small and large intestines.

A. DIFFERENTIAL PARASITE COUNTS OF THE ABOMASUMS.

2. PRINCIPLES

This is a simple and most reliable qualitative technique for isolating, counting and identifying adult nematodes in the abomasums. The main aim of the technique is to determine the level of parasitic infection in addition to the assessment of species present.

3. APPLICATION

- This procedure is used for the isolating, counting and identifying adult nematodes in the abomasums for assessment of parasitic burden in animals

4. OBJECTIVE

- To describe the procedure for isolating, counting and identifying adult nematodes in the abomasums for assessment of parasitic burden in animals

5. APPARATUS

5.1 A tray about 30x45x15cm. The precise size is not important. Suitable plastic trays are easily procurable.

5.2 One or two large, wide-mouth plastic jars or buckets of about 3-5 litres capacity. These are used to collect the contents of each organ examined and hence are called the "total contents jars. Calibrate the sides of the total contents jars in litres.
5.3 A large kitchen ladle or similar utensil with about a 40ml capacity and with a handle about 12 inches long.
5.4 A smaller, wide-mouthed glass or plastic jar of about 500-1000ml capacity. This jar must have a close-fitting screw-top lid. Make a hole in the top of the lid as large as possible, without interfering with proper sealing between the edge of the lid and the top of the jar. Cut and fix a piece of brass wire or nylon mesh (40mesh per linear inch) neatly inside the lid. Calibrate the sides of the "wash jar" in 100ml. gradations. This jar is used to wash the colouring matter out of the faeces and is called the wash jar.
5.5 Two glass petri dishes about 9cm in diameter.
5.6 A saturated aqueous solution of sodium thiosulphate.
5.7 A light box or some white background material. A large white tile is very suitable. Paper will suffice or the bottom of a petri dish can be painted white.
5.8 A mounted needle or fine forceps to handle the worms during counting.
5.9 A jug and a bucket for handling water are useful addition to field equipment, although suitable utensils may be readily procurable from the farmers.
5.10 An illuminated background much eye-strain may result from doing large numbers of worm counts indoors where lighting is poor or variable. An illuminated background overcomes this. Electric lamps, preferably fluorescent are fitted inside a wide shallow box. The top of the box is made from translucent white plastic or ground glass. Samples in clear glass petri dishes are placed on top. The diffuse white light shining up through the petri dishes provides a strong contrast for the stained worms and no shadows are cast

6. REGENTS, SOLUTION AND BUFFER

- An aqueous solution of iodine.

7. PROCEDURE

7.1 During the post-mortem examination, ligate the abomasums with string and separate it from abomasums and duodenum.
7.2 Place the abomasums in a tray. Open the abomasums along the greater curvature so that its contents fall into the tray: empty the abomasums contents into the total content jar.
7.3 Wash the empty abomasums thoroughly in the tray several times, paying particular attention to cleaning between the folds of the mucous membrane. Add the washings to the total contents jar.
7.4 For cattle: make the total volume of the contents and washings in the total contents jar up to 4 litres with water. Occasionally it will be necessary to make the total volume up to 5 litres for cattle. For sheep and goats: make the volume up to 2 to 3 litres.
7.5 Using the large ladle, stir vigorously until all food material, mucous and water are thoroughly mixed.
7.6 Transfer a total of 200ml of the contents to the wash jar in 5 steps of 40ml per step, using the ladle container and stirring the mixture continuously.
7.7 Fill the wash jar with water. Screw the lid on securely. Invert the jar and shake it till the most of the fluid is shaken out. Repeat this process until all faecal coloring matter is removed.

7.8 Add water to make the volume in the wash jar up to 50ml (for convenience).
7.9 Pour small volumes into petri dishes.
7.10 Add a few drops of iodine solutions to the samples in each petri dish. Mix the iodine with the samples and allow to stand for 35 minutes, during which time the worms will stain deeply with iodine.
7.11 Count the number of each species of nematode present in the sample. Repeat the process for each petri dish, and add the species count for all dishes.
NB. In the case of a field post-mortem, procedure 10 to 11 can be conveniently carried out on return to the laboratory.
7.12 **Calculation:** for cattle, multiply the total count for each species by 20 to arrive at the total burden in the animal examined (assuming that an original volume of 2 or 3 litres was used).
7.13 For Haemonchus, small difference in worm burdens may cause significant differences in their pathogenic effect. For this reason, a more accurate assessment of the burden should be obtained by carrying out a total abomassal count of Haemonchus as opposed to the sub-sampling procedure described above.

B. ISOLATING INHIBITED/IMMATURE LARVAE FROM THE ABOMASUMS.

2. PRINCIPLES
- This is a quantitative procedure for isolating, counting and identifying larvae from the intestinal mucous membrane

3. APPLICATION
- This test is used for the isolating, counting and identifying larvae from the intestinal mucous membrane

4. OBJECTIVE
- To describe the process for isolating, counting and identifying larvae from the intestinal mucous membrane

5. APPARATUS
5.1 A tray or bucket
5.2 Normal physiological saline 0.9% (sodium chloride-kitchen salt 9g and distilled water 100ml)
5.3 Nylon net, 32 mm mesh.
5.4 Beaker, petri dishes and wash bottle.
5.5 Pasteur pipette, micro slides and coverslips.
5.6 Microscopes/dissecting microscope.

6. REGENTS, SOLUTION AND BUFFER
- Saline solution

7. PROCEDURE
7.1 Place the opened and washed abomasums with the mucous membrane face down in the tray/ bucket containing lukewarm normal saline.

7.2 Leave the abomasums to soak overnight.
7.3 Remove the abomasums, rinse well with saline solution and discard.
7.4 Pour the saline solution left in the tray/bucket through the nylon net, which will retain the larvae.
7.5 Flush the larvae from the nylon net into a beaker using the wash bottle. Make the total volume up to 200ml.
7.6 Using a dissecting microscope, examine an aliquot of 10ml in a petri dish and count the larvae.
7.7 To identify the parasite species, transfer further sub-samples by Pasteur pipette to microslides for examination under the microscope.
7.8 **Calculation**: The total number of larvae is calculated as:
7.9 (Number in 10ml sub-samples x 20 = Total abomassal larval count)

C. DIFFERENTIAL PARASITE COUNTS OF THE SMALL INTESTINE.

2. PRINCIPLES
- This is a quantitative procedure for isolating, counting and identifying larvae from the intestinal mucous membrane

3. APPLICATION
- This test is used for the isolating, counting and identifying larvae from the intestinal mucous membrane

4. **OBJECTIVE**
- To describe the process of isolating, counting and identifying larvae from the intestinal mucous membrane

5. APPARATUS
5.1 A tray or bucket
5.2 Nylon net, 32 mm mesh.
5.3 Beaker, petri dishes and wash bottle.
5.4 Pasteur pipette, micro slides and coverslips.
5.5 Microscopes/dissecting microscope.
5.6 Gut runner

6. REGENTS, SOLUTION AND BUFFER
- Normal physiological saline 0.9% (sodium chloride-kitchen salt 9g and distilled water 100ml)

7. PROCEDURE
7.1 The procedure used for the small intestine is similar to that for the abomasums.
7.2 When examining the small intestine it is convenient to run the intestine out, free from the mesentery into one tray.
7.3 Initially, the gut is washed by pouring water into one end of the gut and flushing it out into the total volume jar. For further washing and scraping the intestine has to be opened.
7.4 It is important to scrape the mucous membrane in same manner to recover the smaller parasites, especially *Trichostrongylus.*

7.5 Opening and scraping can be done quickly, efficiently and easily in one operation using a simple instrument that can be made by any skilled metal fitter. The instrument is called a gut runner.
7.6 When the small intestine have been opened, scraped and washed, place all of the contents plus of the washings in the total contents jar.
7.7 The procedure for sampling, washing, sub-sampling, staining and counting is the same as previously described for parasites of the abomasums.

Note: Even very large numbers of the smaller nematodes can be very easily overlooked unless some kind of washing procedure is used. They are very difficult to detect when mixed with faecal material.

D. TECHNIQUE FOR DIFFERENTIAL PARASITE COUNTS OF THE LARGE INTESTINE.

2. PRINCIPLES
- This is a quantitative procedure for isolating, counting and identifying larvae from the intestinal mucous membrane**.**

3. APPLICATION
This test is used for the isolating, counting and identifying larvae from the intestinal mucous membrane

4. OBJECTIVE
To describe the process of isolating, counting and identifying larvae from the intestinal mucous membrane

5. APPARATUS
5.1 A tray or bucket
5.2 Normal physiological saline 0.9% (sodium chloride-kitchen salt 9g and distilled water 100ml)
5.3 Nylon net, 32 mm mesh.
5.4 Beaker, petri dishes and wash bottle.
5.5 Pasteur pipette, micro slides and coverslips.
5.6 Microscopes/dissecting microscope.
5.7 Gut runner.

6. REGENTS, SOLUTION AND BUFFER
- Normal physiological saline 0.9% (sodium chloride-kitchen salt 9g and distilled water 100ml

7. PROCEDURE
7.1 Uncoil the large intestine into one tray. Open them with scissors, placing the opened portion into the second tray.
7.2 The nematodes of the large intestines are easily seen. There are relatively few of them and they can be picked off with forceps as the gut is opened and can be placed in a petri dish containing water. Few parasites will be overlooked using this procedure.

7.3 When the contents are fluid because of diarrhoea or when more precise count is required, the counts should be processed as described for the abomasums and small intestine. A large open sieve of 40 mesh/inch brass were can be used.

8. RESULT INTERPRETATION

For the identification of the larvae of domestic animals, some key points are given in the following tables.

Table: Key to the infective larvae of some common nematodes of cattle

1.	Sheath absent, oesophagus more than 1/3 the length of the body. Sheath present, oesophagus short.	*Strongyloides* 2
2.	Length, including sheath, less than 600 µ. Length, including sheath, more than 600 µ	*Bunostomum* 3
3.	Tail of sheath less than 200 µ. Tail of sheath more than 200µ.	4 5
4.	Two conspicuous oval bodies at anterior end of oesophagus. No such structures at anterior end of oesophagus	6 7
5.	Length including sheath more than 1000µ; tail of sheath dorsal and ventral lobes with a rod-like process between. Length including sheath less than 1000µ, tail of Larva ending in a simple point.	*Nematodirus*
6.	Length, including sheath, usually more than 850µ; tail of sheath usually more than 150µ long, gradually to end bluntly.	*Cooperia oncophera*
	Length, including sheath, usually less than 850 µ ; tail of sheath tapering rapidly to a point or short fine filament less than 150 µ long.	*Cooperia punctata* *A. pectinata*
7.	Tail of sheath short and conical, less than 110 µ long. Tail of sheath at least 126µ long	*Trichostrongylus axei* 8
8.	Tail of ending bluntly. Tail of sheath ending in a fine whip-like filament	*Ostertagia ostertagii* *Haemonchus contortus*

Key to infective nematode larvae of sheep and goats

Total length of larva (µ)	**Length, end of larva to end of sheath (µ)**	**Species, with range of total length (µ)**	**Other differential features**
Short 500-700	No sheath 85-115	*Strongyloides* 570-700	Slender body with oesophagus, 1/3 to ½ total length of larvae
Short 500-700	Long 85-115	*Bunostomum* 510-670	Wide body with sudden tapering to long thin tail. “Band” constriction on oesophagus.

Medium 650-900	Short 20-40	*Trichostrongylus* 620-910	Short straight larva, conical tail sheath. Tubercles on tail of larva. Intestinal cells usually prominent.
Medium 650-900	short 20-40	*Ostertagia* 790-910	Long, conical, “finger-like” tail sheath.
Medium 650-900	Short 20-40	*Cooperia curtic* 710-850	Oval bodies at anterior end of larva. Tail of larva rounded.
Medium 650-900	Medium 30-60	*Haemonchus* 650-750	Tail sheath is usually “Kinked” pointed tail of larva.
Medium 650-900	Medium 30-60	*Cooperia onchophera* 800-920	Oval bodies anterior end of larva. Tail of larva rounded.
Long 900-1200	Long 60-80	*Chabertia* 710-790	Stout body with 24 to 32 Rectangular intestinal cells.
Long 900-1200	Long 60-80	*Oesphagostomum* 770-920	Usually longer than Chabertia. Has 16 to 24 traingular intestinal cells.
Long 900-1200	Extremely long 250-290	*Nematodirus* 922-1180	Tail of larva is forked.

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

- N/A

12. REFERENCES

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TITLE: Examination of skin scraping for ecto-parasites

PREPARED: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

At the edge of the cutaneous lesions or predilection sites of ectoparasites like the mange mites are found embedded under skin and sub cutis of animals.

The affected areas usually have wound with oozing fluids and foul smelling. It is clinically noticed with animals' tendency to scratch with whatever hard object they find, which later on the bleeding occurs. While collecting samples for parasite determination, the affected area needs to be scooped till the blood oozes out.
This is a simple qualitative diagnostic technique for the identification of mange mite parasites in various animals.

2. PRINCIPLES

The Sodium or Potassium Hydroxide solution digests the macerates keratin substance of the skin and sets arthropods free leaving the chitinous exoskeletons.

3. APPLICATION

- This procedure is used for examination of skin scrapings for ecto-parasites

4. OBJECTIVE

- To describe the procedure for examination of skin for ecto-parasites

5. APPARATUS

5.1 Scissors and scalper blade
5.2 Glass slides and cover glass
5.3 Collecting containers (glass tubes, small plastic bags, paper etc)
5.4 Water bath, test tube, Centrifuge
5.5 Microscope.

6. REAGENTS, SOLUTION AND BUFFER

- Spirit and 10% Sodium or Potassium Hydroxide solution

7. PROCEDURE

7.1 Direct examination

If no mites can be seen and little materials are available, parasites may be detected by this method. This method is recommended if *Demodex* is suspected.

7.1.1 Place a suitable quantity of the scraped materials on a clean glass slide.
7.1.2 Mix the scraping with oil or water.
7.1.3 Dip on a cover glass and press gently.
7.1.4 Examine carefully with a microscope using low power.

This method is recommended if *Demodex* is suspected as this mite is easily destroyed by prolonged treatment in caustic potash solution.

7.2 Digestion method (Alkali maceration technique)

This method is usually recommended where large amounts of samples are available. The individual or pooled scrapings are placed in a boiling tube and covered with 10% caustic potash and kept in water bath for digestion of tissues.

7.2.1 Place the specimens in a test tube and add 5 ml of 10% potassium hydroxide solution.
7.2.2 Heat gently to boiling point for about 5 minutes or put in water bath till a homogenous suspension is obtained.
7.2.3 Examine a drop of the sediment under a low magnification for mites or fungi.

Permanent mounts are made by first mixing the deposit with water, spinning down and decanting the supernatant, then mixing with the deposit 0.5 ml of melted glycerine jelly. The mixture is aspirated on to slides and covered with cover slips. The jelly sets rapidly and slides may be examined almost immediately using a low magnification. Any mites or suspicious mites are marked and the cover sealed with varnish.

8. RESULT INTERPRETATION

Morphology of parasites demonstrated in annexure

9. WASTE DISPOSAL

- It should be disposed in proper and hygienic way.

10. RISK ASSESSMENT

- Some parasitic infestation or diseases may be of zoonotic nature hence, should be handled with care.

11. TROUBLESHOOTING

- N/A

12. REFERENCES

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

OIE terrestrial manual 2016, chapter 2.9.7

TITLE: Antibody ELISA test for *Fasciola hepatica*

PERPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

Fasciolosis, or Liver Fluke, is a helminthiasis caused by a trematode, *Fasciola hepatica* (or *F. gigantica*, depending on the geographical area), a parasite that settles in the biliary ducts of many different species (ruminants, horses, humans). The clinical signs of this disease in cattle and sheep are anaemia and enteritis resulting, eventually in cachexia. More often, it progresses slowly and presents as a chronic disease.
This herd disease can be rapidly diagnosed by serology using ELISA and easily treated by the appropriate choice of therapy. This ELISA kit (idexx) allows detects the antibodies directed to *Fasciola hepatica*.

2. PRINCIPLES

Liver fluke antibodies are detectable for 12 weeks post infection. This serology test is to determine if the animals have had a liver fluke infestation in the last 12 weeks. To determine if a patent liver fluke infection is present, a parasitology test needs to be done on a faecal sample to look for fluke eggs.

The principle of the test is:

2.1 The wells of the polystyrene microplate are coated with "f2" antigen (only the even-numbered columns are coated with the specific antigen).

2.2 The samples to be tested are diluted and incubated in the wells. Any antibodies specific to "f2" antigen present in the serum will form a "f2" antigen antibody immune-complex and remain bound in the wells.

2.3 After washing, a Peroxidase conjugated anti-ruminant IgG antibody is added to the wells. This conjugate will bind to the immune-complex.

2.4 After another washing, the enzyme substrate (TMB) is added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of the colour is a measure of the rate of antibodies present in the sample to be tested.

3. APPLICATION

- This kit is used to diagnose Fasciolosis in cattle.

4. OBJECTIVE

- To describe procedure for determining the rate of antibodies directed to *Fasciola hepatica*

5. APPARATUS

5.1 Kit Contents

5.1.1	*F. hepatica* f2 antigen coated plates	5
5.1.2	Positive control	1ml
5.1.3	Negative control	1ml
5.1.4	Conjugate concentrate (100X)	0.75ml
5.1.5	Dilution Buffer N.1	120ml
5.1.6	Dilution Buffer N.2	120ml
5.1.7	TMB substrate N.13	60ml
5.1.8	Stop Solution N.3	60ml
5.1.9	Wash Concentrate solution (20X)	100ml

5.2 Materials required but not supplied

5.2.1 Centrifuge (capacity 2000 X g)
5.2.2 Precision Micropipettes and Multi-dispensing micropipettes (reagent volumes listed in the "Test protocol" require pipette precision less than or equal to 5%)
5.2.3 Disposable pipette tips
5.2.4 Micro plate shaker
5.2.5 Distilled water or deionized water
5.2.6 Micro plate washer (manual ,semi-automatic ,or automatic system)
5.2.7 Micro plate covers(lid, aluminum foil or adhesive)
5.2.8 96-well micro plate reader equipped with 450 nm filter
5.2.9 Incubator capable of maintaining a temperature of +37oc (+30c)

6. REAGENTS, SOLUTION AND BUFFER

As mentioned above

7. PROCEDURE

7.1 Wash solution

The wash concentrate (20X) must be diluted 1:20 with distilled / deionized water before use (e.g 15ml of wash concentrate (20X) in 285 ml of distilled water)
This solution is hereafter called "Wash Solution "
Note: The wash concentrate (20X) should be brought to 18-26oc and well mixed to ensure dissolution of any precipitated salts. The wash Solution is stable for up to 3 days when stored at 2-8 ^{o}C.

7.2 Conjugate.

The conjugate concentrate (100X) must be diluted 1:100 in the Dilution buffer N.1.
Note: Diluted conjugate solution is stable for up to 8 hours at 18-26oc.

7.3 Bovine tank milk samples.

Only full fat milk sample should be tested.

7.4 Test Procedure

All reagents must be allowed to come to 18-260 c before use
Reagents should be mixed by gentle swirling. Use a separate pipette tip for each sample
Controls may be dispensed anywhere on the micro plate.
Obtain coated micro plates and record the position of each sample on a work sheet.

7.4.1 Distribution of Samples:

Individual and pool of serum samples

7.4.1.1 Dispense 190µL of Dilution Buffer N2 into each well
7.4.1.2 Dispense 10µL of UNDILUTED Negative control into one appropriate –Ag well (odd numbered column) and into one appropriate +Ag well (even numbered column).
7.4.1.3 Dispense 10µL of UNDILUTED Positive control into two appropriate –Ag well (odd numbered column) and into two appropriate +Ag well (even numbered column).
7.4.1.4 Dispense 10µL of UNDILUTED samples into remaining wells: into one appropriate –Ag well (odd numbered column) and into one appropriate +Ag well (even numbered column).

Tank milk samples (whole milk)

7.4.1.5 Dispense 190µL of Dilution Buffer N2 into three appropriate –Ag wells (Odd numbered column) and into three appropriate +Ag wells (even numbered column)
7.4.1.6 Dispense 10µL of UNDILUTED Negative control into one appropriate –Ag well (odd numbered column) where the dilution buffer N.2 was dispensed and into one appropriate +Ag well (even numbered column).were dilution buffer N.2 was dispensed and into one appropriate +Ag well (evened numbered column) where dilution Buffer N.2 was dispensed.
7.4.1.7 Dispense 10µL of UNDILUTED Positive control into two appropriate –Ag well (odd numbered column) where Dilution Buffer N.2 was dispensed and into two appropriate +Ag well (even numbered column) Where the Dilution Buffer N.2 was dispensed.
7.4.1.8 Dispense 200µL of UNDILUTED samples into remaining wells: into one appropriate –Ag well (odd numbered column) and into one appropriate +Ag well (even numbered column).

7.4.2 Homogenise contents of the wells using a micro plate shaker.
7.4.3 Cover the micro plate and incubate for 1 hour (+ 5 min.) at +370c (+3oc).
7.4.4 Empty the wells of the micro plate and wash with approximately 300 µL of wash solution three to five times .Discard the liquid content of all wells after each wash for serum samples .Following the final wash fluid removal, firmly tap residual wash fluid from each micro plate onto absorbent material. Avoid micro plate drying between washes and prior to the edition of the next reagent.

Note: with full fat milk, it is recommended to modify the method of washing by addition of a soak steps for 1 minute per wash cycle. This facilitates the elimination of fat particles that are

likely to fix in a non-specific way the conjugate in the next step. Thorough washing is essential for optimal results.

7.4.5 Dispense 100 µL of diluted conjugate into each well.
7.4.6 Cover the micro plate and incubate for 30 minutes (+30 min) at +370c (+3oc).
7.4.7 Empty the wells of the micro plate and wash with approximately 300 µL of wash solution three to five times .Discard the liquid content of all wells after each wash for serum samples .Following the final wash fluid removal, firmly tap residual wash fluid from each micro plate onto absorbent material. Avoid micro plate drying between washes and prior to the edition of the next reagent.
7.4.8 Dispense 100 µL of TMB. Substrate N.13 in to each well.
7.4.9 Incubate 20 minutes (+-3 min) at 18-260c in a dark place.
7.4.10 Dispense 100 µL of stop solution N.3 into each well. Shake the micro plate by gentle tapping. Wipe carefully the underside of the micro plate
7.4.11 Blank the micro plate reader on air.
7.4.12 Measure and record optical Densities value of samples and controls at 450 nm.
7.4.13 Calculate Results.

Note : when using robotics, incubation of micro plate in an incubate chamber allows working without plate covers .Use of robots is also not compatible with gentle micro plate tapping or wiping .Plates can be held up to 1 hour in the dark prior to reading .

8. RESULT INTERPRETATION

Calculate the positive control mean ($PC\bar{x}$), positive control mean Net Extinction ($NE\bar{x}pc$) and Negative Control Net Extinction (NENC) value.

$PC\bar{x}= (PC1_{+Ag}\ A_{450}+PC2_{+Ag}\ A_{450})/2$
$NE\bar{x}_{pc=} (PC_{1+\ Ag}\ A_{450}+PC1_{+Ag}\ A_{450}) + (PC2_{+Ag}\ A_{450}- PC2\text{-}A_{450})/2$
$NC_{NC}=NC_{Ag+}A_{450}\text{-}NC_{Ag}\text{-}A_{450}$

For the assay to be valid ,the Positive Control Mean ($PC_{\bar{x}}$) must be greater than or equal to 0.350 optical Density (OD).In addition, the ration between the Positive Control Mean Net Extinction($NE\bar{x}pc$) and the Negative Control Net Extinction(NE_{NC}) can be equal to Zero; in this case, use the absolute Value for the validation .

Note: The Negative Control Net Extinction (NC_{NC}) can be equal to zero; in this case use the of 0.001 to calculate the ratio.

Note; IDEXX has instrument and software system available which calculate means and %blocking and provide data summaries.

Calculation

Calculate the Net Extinction value for each sample by subtracting the corresponding 0D450 value obtain in the control well(S-AG A450) from the 0D450 value obtained in the coat well(S+AG A450).

$NE=S_{+AG}\ A_{450}- S_{-Ag}\ A_{450})$

Calculate the sample to positive(S/P) percentage for each sample:

$S/p\%=100\ \%(\ NE/NE\bar{x}pc)$

Interpretation of Results

Individual sera

Samples with S/P% less than or equal to 30% are considered Negative for the presence of *Fasciola hepatica* Antibodies
Samples with S/P % greater than 30% and less than or equal to 80% considered Mild Positive for the presence of *Fasciola hepactica* Antibodies.
Samples with S/P% greater than 80% and less than 150% are considered positive for the presence of *Fasciola hepatica* Antibodies.
Samples for S/P % greater than or equal to 150% are considered strong positive for the presence of *Fasciola hepatica* Antibodies.

Pool of sera & Tank Milk

Samples with S/P % less than or equal to 30% are considered negative for the presence of antibodies against *Fasicola hepatica*.
Samples with S/P % greaten then 30% are considered positive for the presence of antibodies against *Fasciola hepatica*.

Notes:

Interpretation on pool of sera and the Tank Milk are only indicative; final interpretation depends on S/P obtained with corresponding Individual samples
The correlation between S/P% levels and infestation levels for individual sera and pool of sera or Tank Milk may be interpreted though a scale of crosses ranging from 0 to +++ as described in the table below:

S/P% of the samples	**Correlation between the test result and infestation level(individual sera)**		**Correlation between test result and the prevalence of infestation within the herd(pools of sera or Tank Milk)**	
% s/p ≥ 150	strong positive for the presence of *Fasciola hepatica* Antibodies	+++	strong infestation(>50% of the infestation)	**+++**
80%<%<150	positive for the presence of *Fasciola hepatica* Antibodies	++	Medium infection (between 20% and 50% of infestation	**++**
30<%S/p≥80	positive for the presence of *Fasciola Hepatica* Antibodies	+	low infestation(<20% of infestation)	**+**
% S/P ≤ 30	Negative for the presence of *Fasciola hepatica* Antibodies	0	No or very weak infestation	**0**

A positive Serology after the return in cowshed reveals the presence of infected animals and pasture land .Even correctly treated with an efficient product, the animal will remain carrier of antibodies during about 12 weeks (this delay may vary according to initial antibody rate).
An animal treated in autumn (in the return in Cowshed) cannot be differentiated from an untreated animal before about 12 weeks .On the other hand, a late serology (at the beginning of the spring) will enable to estimate the efficiency of a treatment.

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

10.1 Susbstrate solution may be irritant to skin and mucus. In case of contact with this solution, rinse thoroughly with water and seek medical attention,

10.2 Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available. The control material is traceable to reference sera panels internally validated.

11. TROUBLESHOOTING

- Clean pipette tips must be used for every assay step. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.

12. REFERENCES

Idexx Fasciolosis verification 06-05120-07

TITLE: Examination of Haemo-parasites by direct smear

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
Various parasitological techniques are used for both clinical diagnosis and to certain extent for preliminary survey of prevalence for haemo-parasitic infections in animals. The techniques using Giemsa stain, Leishman stain and wet blood smear examination and lymph node aspirate examination are some of the simple and common laboratory techniques to facilitate the diagnosis and recognition of haemo-parasitic infections in animals.

2. PRINCIPLES
The thick blood films are prepared from whole blood. After staining, the slide is examined under the microscope.

3. APPLICATION
This test is used for the diagnosis of haemo-parasitic infection in animals

4. OBJECTIVE
- To describe the procedure for the diagnosis of haemo-parasitic infection in animals

5. APPARATUS
5.1 Glass slides and covers glass.
5.2 Hemotocrit tube and Microhaematocrit centrifuge.
5.3 Microscope

6. REAGENTS, SOLUTION AND BUFFER
6.1 Giemsa solution
6.2 Leishman's stain solution
6.3 Methanol
6.4 PBS

7. PROCEDURE
7.1 Blood examination using Giemsa stain
7.1.1 Place a drop of blood on one end of a microscope slid and draw the blood into a thin film.
7.1.2 Air-dry the film; protect the blood film from flies and other insect if it is not to be stained immediately.
7.1.3 Fix in absolute methanol for 5 minutes.

7.1.4 Air –dry.
7.1.5 Dilute stock Giemsa stain 1:9 with PBS (6.4 – 6.8pHh for bovine and other species except pH 5.6 for avian species) and flood the film (or place slide in staining jar). Fresh stain should be prepared at least every 2 days.
7.1.6 Stain for 45 minutes
7.1.7 Wash stain away gently with distilled water.
7.1.8 Air-dry, parasites will stain with blue cytoplasm and magenta nuclei.

7.2 Blood examination using Leishman stain
7.2.1 Stain the dried blood stain slide with 1 ml of Leishman's solution for 1 minute.
7.2.2 Dilute the stain over the smear with double the quantity of distilled water and mix well 9 Buffer solution of PH 6.8 is preferred)
7.2.3 Allow the diluted stain to act for 10-15 minutes and wash with distilled water.
7.2.4 Treat the slide with PH6.8 buffer for 2 minutes.
7.2.5 Rinse quickly with distilled water, shake off the excess water.
7.2.6 Blot and dry carefully with fibre free blotting paper.
7.2.7 Clear ad mount in neutral mounting medium.
7.2.8 Examine with oil immersion objective.

7.3 Wet blood smears technique
Drying and fixing of blood smears leads to some alteration of the morphology of intra erythrocyte parasites. This technique overcomes the problem of biconcavity of erythrocytes so that intra erythrocyte parasites can be visualized because refraction of light induced by the biconcavity of bovine erythrocytes makes it difficult to appreciate the morphology of intracellular, unstained parasites. In a hypotonic environment, an erythrocytes swells to its maximum and becomes a sphere and the parasites and associated structures become visible within the cells. Stained hemoglobin appears to mask intracellular inclusions associated with parasites (eg. *Theileria orientalis*) which are visible in wet blood smears.
7.3.1 A small drop blood enough to give a single layer distribution of erythrocytes id taken on a glass slide and missed with a smaller drop water saline using a glass slide.
7.3.2 A clean cover slip is gently placed over the preparation avoiding the formation of air bubbles.
7.3.3 Excess blood is absorbed using a piece of blotting paper.
7.3.4 The smear is then to be examined under oil immersion.

7.4 Lymph node aspirate examination
7.4.1 The swollen lymph node (usually pre-scapular, parotid and pre-crural) is cleaned with alcohol.
7.4.2 The lymph node is held in between the index finger and thumb firmly and the sterilised 16 to 18 gauge needle is slowly slipped in (0.2 ml of fluid is withdrawn and spread on clean slide.
7.4.3 The fluid is air dried and stained with one the Romanowsly's stain.
7.4.4 Trypanosomiasis (extra cellular parasites) and Theileriosis (Koch's blue bodies) can be identified by this examination.

8. RESULT INTERPRETATION

Results are interpreted as per the standard identification protocol described in parasitology manuals

9. WASTE DISPOSAL

- Since the blood may contain infectious pathogens, it should be disposed properly.

10. RISK ASSESSMENT

- Blood may contain infectious pathogens that may infect humans.

11. TROUBLESHOOTING

- During staining, thin blood films are essential if artefacts are to be reduced to a minimum. They should be dried quickly in air and if they are to be kept for any period before staining they should be fixed in methyl alcohol

12. REFERENCES

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FAO (1998). A field guide for the diagnosis treatment and prevention of African animal typanosomiasis Pp.141-149.

Peter Deplazes, Johannes Eckert, Alexander Mathis, Georg von Samson-Himmelstjerna and Horst Zahner (2016). Parasitology in Veterinary Medicine. Wageningen Academic Publishers.

TITLE: Parasitological examination of Microfilaria in blood

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

Microfilaria is a term used to describe the juvenile stage of any parasite that lives in the host's circulatory system. This term is used most frequently in relation to heartworm disease. Heartworms invade the circulatory system of mammals, primarily dogs, and live off the host animal until the parasite dies and is absorbed by the host's body.
Simple blood tests using different methods can be performed on companion animals to detect microfilaria.

2. PRINCIPLES

In patients infected with filarial parasites, adult worms live in the lymphatic system, subcutaneous tissues and deep connective tissues. The adult female produces microfilaria which can be detected in peripheral blood. Thin and thick blood smears are prepared and stained with Wright's and Giemsa stains. A concentration procedure is also performed since the number of circulating microfilaria may be very low. The concentrated sediment is examined microscopically.

3. APPLICATION

- This test is used for the Parasitological examination of Microfilaria in blood

4. OBJECTIVE

- To describe the procedure the Parasitological examination of Microfilaria in blood

5. APPARATUS

5.1 Syringes,
5.2 Hypodermic needles (for collection of blood),
5.3 Glass sliders,
5.4 Cover slips,
5.5 Microhematocrit tubes,
5.6 Hematocrit centrifuge
5.7 Microscope

6. REAGENTS, SOLUTION AND BUFFER

6.1 0.1% methylene blue
6.2 2% formalin
6.3 Oil immersion

7. PROCEDURE

7.1 Microfilaria Detection

This is the simplest and most rapid of the procedures to be described for microfilaria; it is not a very sensitive technique but can be used to evaluate the pattern of movement of microfilaria when attempting to differentiate between *Dirofilaria* and *Dipetalonema*.

7.1.1 Place one drop of venous blood onto a clean microscope slide.
7.1.2 Place a cover slip over the drop of blood.
7.1.3 Examine the cover slip area under low magnification of the microscope. Look for the undulating movements of the larvae, which may retain their motility for as long as 24 hours.

7.2 Hematocrit test

This technique is only slightly more sensitive than the direct smear and it not used extensively.

7.2.1 Draw fresh whole blood into a microhematocrit tube, as for a routine packed-cell volume test.
7.2.2 Spin for 3 minutes in a hematocrit tube centrifuge.
7.2.3 Examine the plasma portion of the separated blood, while still in the tube, under low magnification (x100). Swimming microfilaria will be present in the plasma above the buffy coat.

The direct smear and microhematocrit techniques may not detect infections with small numbers of microfilaria. Therefore, for use as a screening procedure for heartworm infection, one of the following concentration techniques is preferred.

7.3 Modified Knott's test

The Knott's technique is a concentration method for the detection of microfilaria in blood. It is generally considered the preferred technique for heartworm screening because it is standard, quick, and index.

7.3.1 Draw a sample of blood into syringe containing anticoagulant such as EDTA or heparin.
7.3.2 Mix 1 ml of the blood with 9 ml of 2% formalin solution. If not well mixed, the red cells will not be thoroughly lysed, making the test much more difficult to read. Microfilaria, but not red cells, will be fixed by 2% formalin. If 10% formalin is used (the concentration used for fixation of tissue) red cells will also be fixed.
7.3.3 Centrifuge the mixture at 1200 rpm for 5 minutes and discard the supernatant fluid.
7.3.4 Add 1 drop of 0.1% methylene blue to the sediment, mix well and transfer the stained sediment to a microscope slide using a Pasteur pipette to collect the entire sample.
7.3.5 Examine under low magnification(x10) microscope. Microfilaria will be fixed in an extended position with nuclei stained blue.

7.4 Filter test

An alternative to the Knott's test is a concentration technique in which microfilariae are trapped on a filter that is then examined under the microscope. This technique can be performed more quickly than the Knott's test, but microfilaria are not as easily measured.

Kits containing filter apparatus, filters, lysing solution, and stain are commercially available. Use of 2% formalin is recommended since other lysing solutions may cause significant changes in the size of microfilariae.

7.4.1 Mix 1 ml of blood with 9 ml lysing solution in a syringe.
7.4.2 Attach the syringe to the filter holder containing a filter with 1µm pore size and empty the syringe.
7.4.3 Fill the syringe with water and pass it through the filter to wash away remaining small debris.
7.4.4 Fill the syringe with air, reattaching to the filter apparatus, and express.
7.4.5 Unscrew the filter assembly, remove the filter with forceps, and place the filter on a microscope slide.
7.4.6 Add 1 drop of 0.1% methylene blue, cover slip, and examine at low power microscope.

8. RESULT INTERPRETATION

Detection of microfilaria in blood confirms a diagnosis of filarial infection.

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

11.1 If parasite(s) were reported and the sample was negative for parasites
  11.1.1 The wrong slide was reviewed
  11.1.2 Identified an element as a parasite
  11.1.3 Review the slide again and refer to reference textbooks to review the morphology of the parasite you thought you observed on the slide.
11.2 If no parasite(s) were reported and the sample was positive for parasite(s)
  11.2.1 The sample was stored at the wrong temperature upon receipt
  11.2.2 The blood film wasn't scanned on a low power objective before going to a high power objective.
  11.2.3 First screen the smear at a low magnification (10× or 20× objective), to detect large parasites (microfilaria) then examine the
  11.2.4 Smear using oil immersion objective. CLSI recommend scanning at least 300 oil immersion fields for the determination of "No Parasite Seen".
  11.2.5 The sample may have contained a low concentration of parasite.

12. REFERENCES

Margaret, W.S Russell, L.K and Anne, M.Z (1994). Veterinary Clinical Parasitology American Association of Veterinary Parasitologiest. The Iowa State University Press, Iowa 50010, USA. Pp.101-120. FAO (1998) A field guide for the diagnosis treatment and prevention of African animal typanosomosis Pp.141-149.

TITLE: Urine sedimentation test for nematodes

PREPARED: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
Some nematodes are parasites of kidney, urinary bladder, and ureters e.g. *Dictophyma renale* and *Stephanurus dentatus* from swine and *Capillaria plica* from cats, dogs and other carnivores. These worms produce eggs which pass in the urine. Through collection of urine and sedimentation, the eggs of parasites could be recovered, identified and treatment regime can be fixed accordingly.

2. PRINCIPLES
This simple sedimentation technique is a qualitative test for the detection of nematode eggs in the urine and concentrating them by means of centrifugation.

3. APPLICATION
- This test is used for presences of nematode eggs in the urine of the animals.

4. OBJECTIVE
- To describe the procedure for diagnosing the presences of nematode eggs in the urine of the animals

5. APPARATUS
5.1 Sterilized urine collection tubes
5.2 Glass sliders
5.3 Cover slips
5.4 Centrifuge
5.5 Microscope.

6. REAGENTS, SOLUTION AND BUFFER
- N/A

7. PROCEDURE
7.1 Collect urine samples in test tubes.
7.2 Transfer the samples to a centrifuge table and centrifuge at 1500 rpm. For 2 to 3 minutes.
7.3 Pour off the supernatant.
7.4 Remove some of the sediment and place on a glass slide (microscopic slide).
7.5 Apply a cover glass and examine under the microscope.

8. RESULT INTERPRETATION

Results are interpreted as positive or negative.

9. WASTE DISPOSAL

- Dispose the urine sample properly in hygienic way.

10. RISK ASSESSMENT

- The urine may contain zoonotic pathogens like *Leptospira, Brucella* etc, which can infect humans hence, utmost care should be taken.

11. TROUBLESHOOTING

- N/A

12. REFERENCES

Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food (MAFF/ ADAS) London, Her Majesty's Stationary Office (1971) Reference Book 418.

TITLE: Detection of *Trichinella spiralis* larvae from muscles

PREPREAD BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.O6.2018

1. INTRODUCTION

Moderate to heavy *Trichinella spiralis* infection can be diagnosed by simply squashing bits of muscle tissue between two glass slides and scanning under low power microscope. The diaphragm and masseter muscles are especially likely to yield positive findings.

Pepsin digestion is used to detect light infection with *T. spiralis* and other nematodes in tissue. Gastric juices digest the muscle tissue but not the larva of *T. spiralis*.
There are various published procedures for the pooled digestion technique for the detection of *trichinella* in meat. Among the techniques, magnetic stirrer method is considered the gold standard technique. According to the current legislation of the EU, artificial digestion methods are acceptable and used for *trichinella* inspection in meat.

2. PRINCIPLES

The muscle larvae are released after digestion of muscle tissues by means of artificial digestion using fluid composed of pepsin and hydrochloric acid. The digestion is then followed by selective screening, filtration or sedimentation procedures and a final microscopic examination for the presence of larvae.

3. APPLICATION

- This test is used for Detection of *Trichinella spiralis* larvae from muscles

4. OBJECTIVE

- To describe the procedure for Detection of *Trichinella spiralis* larvae from muscles

5. APPARATUS

5.1 Pepsin digestion method

5.1.1 Blender jar,
5.1.2 Magnetic stirrer,
5.1.3 Test tube,
5.1.4 Petri dish,
5.1.5 Pasture pipette
5.1.6 Stereo zoom microscope.

5.2 Artificial digestion technique/Magnetic stirrer method – As per regulation EC-2075/2005

5.2.1 Knife or scissors and tweezers.

5.2.2 Trays marked off into 50 squares, each of them can hold samples of approximately 2 g of meat, or other tools to ensure the traceability of the samples.
5.2.3 A blender with a sharp chopping blade. If the samples are larger than 3g a meat mince with openings of 2mm to 4mm or scissors must be used. In the case of frozen meat or tongue (after removal of the superficial layer, which cannot be digested) a meat mincer is necessary and the sample size should be increased considerably.
5.2.4 Magnetic stirrers with thermostatically controlled heating plate and Teflon-coated stirring rods approximately 5 cm long.
5.2.5 Conical glass separation funnels, capacity of at least 21 preferably fitted with Teflon safety plugs.
5.2.6 Stands, rings and clamps.
5.2.7 Sieves, mesh size 180 um, external diameter 11 cm with stainless steel meshes.
5.2.8 Funnels, internal diameter not less than 12 cm, to support the sieves.
5.2.9 Glass beakers, capacity 31.
5.2.10 Glass measuring cylinders, capacity 50 ml to 100 ml or centrifuge tube.
5.2.11 A trichinoscope with a horizontal table or a stereo-microscope, with a sub stage transmitted light source of adjustable intensity.
5.2.12 A number of 9cm diameter Petri dishes for use with a stereo-microscope marked on their undersides into 10mm x10 mm square examination areas using a pointed instrument.
5.2.13 A larval counting basin (for use with a trichinoscope), made of 3mm thick acrylic, plates as follows; the bottom of the basin should have 180 mmx40mm, marked off into squares the sides 230 mmx20 mm. The end 40mmx20mm. The bottom and the ends must be inserted between the sides to form two small handles at the ends. The upper side of the bottom is raised 7 mm to 9 mm from the base of the frame formed by the side and the ends. The components must be stuck together with glue suitable for the material.
5.2.14 Aluminium foil,
5.2.15 25% hydrochloric acid.
5.2.16 Pepsin, strength: 1:10,000 NF (USA National formulary) corresponding to 1: 12,5000BP (British Pharmacopoea) and to 2,000 FIP (Federation internationale de pharmacie).
5.2.17 Tap water heater to 460C to 480C.
5.2.18 A balance accurate to at least 0.1g
5.2.19 Metal trays, capacity 10 L to 15 L, to collect the remaining digestive juice,
5.2.20 Pipette of different sizes (1 ml, 10 ml and 25 ml) and pipette holders,
5.2.21 A thermometer accurate to 0.50C within the range 10C to 1000C.
5.2.22 Siphon for tap water.

6. REAGENTS, SOLUTION AND BUFFER

Pepsin digestion method

6.1 Sol. 1 (1% w/v pepsin-1gm of pepsin in 99ml of water),
6.2 Sol. 2 (1ml of con.HCL in 99 ml of dist. Water),

7. PROCEDURE

7.1 Sampling

7.1.1 In the case of whole carcasses of domestic swine a specimen weighing at least 1 g has to be taken from a pillar of the diaphragm at the transition to the sinewy part. Special trichinae forceps can be used if an accuracy of 1 g to 2.15 g can be guaranteed.

7.1.2 In the case of breeding sows and boars, a large sample weighing at least 2g has to be taken from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimens of twice the size 2 g for 4 g in the case of breeding sows and board) had to be taken from the rib part or from the jaw muscle, tongue or abdominal muscles.

7.1.3 For cuts of meat, a sample weighting at least 5 g of started muscle, containing little fat has to be taken if possible form close to bones or tendons. A sample of the same size has to be collected from meat that is not intended to be cooled thoroughly or other types of past-slaughter processing.

7.1.4 For frozen samples, a sample weighing at least 5 g of striated muscle tissue has to be taken for analysis. The weight of meat specimens relates to a sample of meat that is free of all fat and fascia.

7.2 Pepsin digestion method

7.2.1 Take about 5gms of Tongue, masseter (cheek) muscle, abdominal muscle and diaphragm. About 20 mgs of sample is to be mixed with 200ml of pepsin solution.

7.2.2 Mix the sample in a blender jar for better digestion.

7.2.3 Heat the sample at 37ºC and mix with pepsin.

7.2.4 Stir the mixture on a magnetic stirrer for 3-4 hrs.

7.2.5 Allow it to settle it for 15-20 mins, aspirate the upper two third of the supernatant by using pasture pipette.

7.2.6 Allow the remaining to settle down again for 15-20 mins and then aspirate the supernatant without disturbing the sediments.

7.2.7 Wash the sediments using warm (37ºC) distilled water and allow to settle down once again for 15-20mins.

7.2.8 Repeat it till the supernatant becomes clear.

7.2.9 Transfer the sample to 50ml tube to settle, aspirate the sediment to desired volume.

7.2.10 Pour the sediment on to a Petri dish and examine under stereo zoom microscope for *T. spiralis* larvae.

7.3 Protocol for artificial digestion technique/magnetic stirrer method – As per EC regulation no. 2075/2005

7.3.1 Complete pools (100 g of samples at a time)

7.3.2 Add 16ml ± 0.5 ml of hydrochloric acid to a 3litre beaker containing 2litre of tap water, preheated to 40^0C to 49^0C, a stirring rod is placed in the beaker, the beaker is placed on the preheated plate and the stirring is started.

7.3.3 Add 10g ± 0.2g of pepsin.

7.3.4 Collect 100g of samples and chop in the blender.

7.3.5 Transfer the chopped meat to the 3L beaker containing the water, pepsin and hydrochloric acid.
7.3.6 The mincing insert of the blender is immersed repeatedly in the digestion fluid in the beaker and the blender bowl is rinsed with a small quantity of digestion fluid to remove any meat still adhering.
7.3.7 Thereafter, cover the beaker with aluminium foil.
7.3.8 The magnetic stirrer must be adjusted so that it maintains a constant temperature of 44^0C to 46^0C throughout the operation. During stirring the digestion fluid, remove any meat still adhering.
7.3.9 The digestion fluid is stirred until the meat particles disappears (approx.30 min).The stirrer is then switched off and the digestion fluid is poured through the sieve into the sedimentation funnel. Longer digestion times may be necessary (not exceeding 60 min) in the processing of certain types of meat (tongue, game meat, etc).
7.3.10 The digestion process is considered satisfactory if not more than 5% of the starting sample weight remains on the sieve.
7.3.11 Allow the digestion fluid to stand in the funnel for 30 min.
7.3.12 After 30 min, run off 40 ml sample of digestion fluid quickly into the measuring cylinder or centrifuge tube.
7.3.13 The digestion fluids and other liquid waste should be kept in a tray until reading of the results is completed.
7.3.14 Allow the sample to stand for 10 min and then carefully withdraw 30 ml of supernatant by suction to remove the upper layer and leave a volume of not more than 10ml.
7.3.15 The remaining 10 ml sample of sediment is poured into a larval counting basin or Petri dish.
7.3.16 The cylinder or centrifuge tube is rinsed with not more than 10 ml of tap water, which has to be added to the sample in the larval counting basin or Petri dish, subsequently, the sample is examined by trichinoscope or stereo-microscope at a 15 to 20 times magnification. In all cases of suspect areas of parasite-like shapes, higher magnification of 60 to 100 times is used.
7.3.17 Digests have to be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

8. Result interpretation

Larvae can be directly counted under a dissecting microscope, or the larva can be retrieved by suction with Pasteur pipette for detailed studies.

8.1 If examination of a collective sample produces a positive or uncertain result a further 20g sample must be taken from each pig. The 20 g samples from five pigs are pooled and examined using the method described above. In this way samples from 20 groups of five pigs will be examined.
8.2 If *Trichinella* is detected in a pooled sample from five pigs, further 20 g samples must be collected from the individual pigs in the group and each is examined separately using the method described above.

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

- Since it is zoonotic parasite, proper care should be taken during handling and disposal of wastes.

11. TROUBLESHOOTING

If the digests are not examined with in 30 min of preparation, they must be clarified as follows.

11.1 The final sample of about 40 ml is poured into a measuring cylinder and allowed to stand for 10 min, and 30ml of the supernatant fluid is then removed, leaving a volume of 10 ml. This volume is made up to 40 ml with tap water. After a further setting period of 10 min, 30 ml of the supernatant fluid is withdrawn by suction leaving a volume of no more than 10 ml for examination in a Petri dish or larval counting basin. The measuring cylinder is washed with no more than 10 ml of tap water and these wasting are added to the sample in the Petri dish or the larval counting basin for examination.

11.2 If the sediment is found to be unclear on examination, the samples is poured into a measuring cylinder and made up to 40ml with tap water and then the above procedure is followed. The procedure can be repeated two to four times until the fluid is clear enough for a reliable reading.

Pools of less than 100g

11.3 If necessary, up to 15g can be added to a total pool of 100g and examined together with these samples.

11.4 More than 15g must be examined as a complete pool. For pools of up to 50g the digestion fluid and the ingredients may be reduced to 1L of water, 8ml of hydrochloric acid and 5g of pepsin.

12. REFERENCES

FAO/WHO/OIE Guidelines for the surveillance, management, prevention and control of TRICHINELOSIS (2007). World Organization for Animal Health (OIE) 12, rue de Porny, 75017 Paris, France. P87-91.

TITLE: Preputial Test for Identification of *Trichomonas*

PREPARED BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION
A protozoan organism, *Trichomonas foetus (Trichomonas foetus)*, is the etiological cause of Trichomoniasis. This organism lives in the reproductive tract of the cow and in the sheath of the bull's penis. It causes infertility in cows.
Bulls will show no signs of the disease but can shed the organism indefinitely. To diagnose the disease, a preputial fluid sample is taken from the sheath of the bull's penis and examined.

2. PRINCIPLES
This is a qualitative test for the detection of Trichomonas parasite from preputial fluid of male organ. It is a direct method consisting of flushing the perpetual fluid from bulls using saline and examined for the presence of parasite using microscope.

3. APPLICATION
This is reasonably a good method for detecting the *Trichomona*s parasite by direct microscopic examination. This diagnostic technique can be used for clinical diagnosis of Trichomoniasis in bulls in field situations.

4. OBJECTIVE
- To describe the procedure for the diagnosis of *Trichomonas* infection in bulls.

5. APPARATUS
5.1 50 ml syringe, pipette (plastic insemination sheath-split end cut off about 4cm)
5.2 Hand gloves
5.3 Universal bottle
5.4 Glass slides
5.5 Cover slips
5.6 Centrifuge
5.7 Microscope

6. REAGENTS, SOLUTION AND BUFFER
- 25 ml sterile buffered saline (pH 7.2).

7. PROCEDURE
7.1 Take the warmed saline solution into syringe and attach plastic pipette.

7.2 Introduce the pipette into the preputial cavity to a distance of about 30cm.
7.3 The assistant now holds the syringe while the operator seals the perpetual orifice with fingers of one the hand.
7.4 The assistant injects the saline into the perpetual cavity, detaches the syringes and closes the free end of the pipette by finger pressure.
7.5 The operator briskly massage the fluid within the cavity with his free hand while maintain fixation of the preputial ring to prevent loss of fluid. At least ten vigorous massage movements should be carried out.
7.6 On completion of massage by the operator, the assistant puts the free end of the pipette into a sterile bottle slightly larger volume, while reopening the closed end by manipulation – a squeezing action.
7.7 The assistant gradually withdraws the pipette, allowing the saline to flow into the bottle. The flow will continue as the pipette is withdrawn deliberately and progressively as directed by the operator. The fluid return can be helped by the operator massaging towards the perpetual orifice, while preventing the escape of fluid between the perpetual ring and the pipette.
7.8 The perpetual washing is spun in a centrifuge at 350g for 10 minutes. The supernatant fluid is discarded.
7.9 A drop of the centrifuged deposit is placed on a microscope slide and the whole area under an 18 x 18 mm cover slip is carefully examined. Any motile protozoa should be checked for the presence of anterior flagella and undulating membrane. The presence of the latter is almost diagnostic.

8. RESULT INTERPRETATION

Results are interpreted as "*Trichomonas* spp. positive, "*Trichomonas* spp. negative", and "suspect for *Trichomonas* spp."

9. WASTE DISPOSAL

- The waste should be disposed properly.

10. RISK ASSESSMENT

- The preputial washings may contain infectious pathogens that may infect humans. Hence, should be handled carefully.

11. TROUBLESHOOTING

NA

12. REFERENCES

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McKenzie RA (ed) (1992). Veterinary Laboratory Users Guide (VLUG), DPI Booklet QI92027, Brisbane, iv + 137pp. ISBN 0 7242 3910 3.

Vaughan J (1993) Bovine Trichomoniasis In Corner, LA and Bagust, TJ (ed) Australian Standard Diagnostic Techniques for Animal Diseases, Standing Committee on Agriculture and Resource Management, CSIRO Information Services, East Melbourne

Dennis M. Mccurnin, Joanna M. Bassert. Clinical textbook for veterinary technicians. Sixth Edition.

TITLE: Identification of Ticks

PREPREAD BY: Parasitology section

REVISED BY: Parasitology section

APPROVED BY: Head LSU

DATE: 11.06.2018

1. INTRODUCTION

All ticks are external blood-sucking parasites of land vertebrates. A wide range of hosts is infected including reptiles, birds, mammals and man. They cause illness themselves as well as being efficient carries in the transmission of a variety of viral, rickettsial, bacterial and protozoan diseases in man and other animals. Therefore, the ability to identify ticks is important in assessing the risk of diseases to livestock.

Ticks are Arachids, dorso-ventrally flattened and body not divided into distinct head, thorax and abdomen. Identification of ticks is made possible by morphological examination of body parts like structure of mouth parts. However, accurate identification of ticks to the species, particularly of the larval and nymphal stages requires specialist knowledge and equipments.

2. PRINCIPLES

The main principle of the technique is for the identification of adult ticks by studying the morphology of body parts using stereo-zoom microscope. This is a qualitative technique for identification of ticks to genus level.

3. APPLICATION

- This process is used identification of adult ticks by studying the morphology of body parts using stereo-zoom microscope

4. OBJECTIVE

- To describe the process of identification of adult ticks by studying the morphology of body parts using stereo-zoom microscope

5. APPARATUS

5.1 Tick samples (with intact body parts so that all features used to identify them are undamaged).
5.2 Petri dishes or any suitable sample holder.
5.3 Hand held lens or a pocket magnifier (for field use).
5.4 Stereo-zoom microscope or stereo microscope.

6. REAGENTS, SOLUTION AND BUFFER

- 60-70% alcohol

7. PROCEDURE

7.1 Take out a tick sample from sample bottle and placed on petri dish or any suitable sample holder.

7.2 Placed the sample under stereo-zoom microscope and examine for morphological features using the key for identification of ticks to genera.

7.3 Each sample should be examined one at a time. To get a good result 25 to 500 tick samples should be examined.

7.4 After examination tick samples should be placed to empty sample container for further examination.

In field conditions tick identification should be carried out using hand lens or pocket-magnifier

8. RESULT INTERPRETATION

Ticks are identified to genus using the shape of their capitulum and length of their mouthparts, the presence or absence of markings on the scutum, and body structures.

9. WASTE DISPOSAL

- NA

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

- The specific identification of ticks can be challenging, particularly if dealing with larval or nymphal forms.

12. REFERENCES

Dennis M. McCurnin & Joanna M. Bassert. Clinical textbook for veterinary technicians. Sixth Edition.

Lora Rickard Ballweber. The Practical Veterinarian. Veterinary Parasitology.

TITLE: Recovery and examination of Metacercaria from herbage

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DATE: 11.06.2018

1. INTRODUCTION

The concentration of metacercariae on the herbage may be determined per unit area or per unit dry weight of herbage. In either case a random sampling procedure should be used (see 5D). Individual sample should not weigh more than 100g (or 20g dry matter).The herbage is cut with curved scissors as close to the ground as possible taking care to avoid extraneous debris. The sample is put in a suitable container and immediately fixed by the addition of 10%formalin.Immediate fixation prevents infected snails (if accidentally included) from liberating additional cercariae as well as permitting the storage of samples and render the metacercariae resistant to the subsequent treatment.

2. PRINCIPLES

This is a qualitative and semi-quantitative procedure for isolation identification and concentration of metacercaria on the herbage per unit area or per unit dry weight of herbage.

3. APPLICATION

- This procedure is used for recovery and examination of Metacercaria from herbage

4. OBJECTIVE

- To describe the procedure for recovery and examination of Metacercaria from herbage

5. APPARATUS

- Scissor and collecting bags
- Buckets, beaker (250ml)and Petri(marked with parallel liner 5mm apart)
- Wire mesh screen with aperture of 0.25mm which rests on a stainless steel (quality FMB) wire mesh screen with an aperture of 0.075mm.
- Vacuum pump and weighing balance.
- Dissecting microscope or microscope.
- Tape water.

6. REAGENTS, SOLUTION AND BUFFER

- Conc. sulphuric acid.
- 10%formalin
- Saturated copper sulphate solution

7. PROCEDURE

- The herbage is cut up into lengths of less than 5 cm. It is vigorously agitated in water to detach the cysts from the herbage. A liquidiser is suitable, running at a moderate speed for 1minute.

- After agitation the material, together with washing from the containers used, is poured through a wire-mesh screen with an aperture of 0.25 mm which rests on a stainless steel (quality FMB) wire –mesh screen with an aperture of 0.075mm, and vigorously washed with a jet of water. The herbage retained by the top screen is dried and weighed. The cysts together with detritus are retained on the lower screen. They are washed to one edge of the screen, allowed to drain thoroughly and transferred, as dry as possible, to a 250ml beaker the screen is set on one side.

- 150ml of concerted sulphuric acid is carefully added, the mixture being stirred mean while. The acid oxidises much of the organic matter and reduces its particles size but the formalized cysts withstand this treatment. The mixture is allowed to stand for 10 minutes (longer exposure of the cysts is not harmful), and is then cautiously added to an equal volume of saturated copper sulphate solution. This limits the action of the acid on the stainless steel screen.

- The whole is now poured through the original screen and any material which was left on the screen in step 2 is thus not lost. Preferably this procedure should be carried out in a sink through which there is a constant water flow. The material retained by the screen is rinsed gently and then washed back into a bowl from which it is transferred to a flask.

- The flask is connected to a vacuum pump and evacuated. This, by removing adhering examined in a Petri dish (marked with parallel lines 5mm apart), with a dissecting 15x wide-angle eye pieces. The cysts are counted. The colour varies from white to brown and occasionally the wall is ruptured and the metacercariae absent. The average recovery rate of the method is 90%.

8. RESULT INTERPRETATION

The metacerceria is identified based on their morphological characteristics.

9. WASTE DISPOSAL

- Follow the SOP of waste disposal

10. RISK ASSESSMENT

- N/A

11. TROUBLESHOOTING

- The test requires lots of time and labour to collect pasture samples
- In the step 4 great care should there to avoid a fierce reaction between sulphur acid and water

12. REFERENCES

Couvillion C. E. 1993. Estimation of the numbers of T*richostrongylid* larvae on pastures. *Veterinary Parasitology* 46:197-203.

Crofton H. D. 1954. The ecology of the immature phases of *Trichostrongyle* parasites. V. The estimation of pasture infestation.

TEST CATEGORIZATION FOR PARASITOLOGY

Sl. No.	Procedure / SOP	DVL	SVL/ TVH	RLDC/ NVH	NCAH
1	Direct technique - Direct wet mount technique (faecal) - Impression/intestinal scrap smear	X	X	X	X
2	Qualitative tests - Floatation technique/simple test-tube floatation test, taeniid egg - Sedimentation test update with modified method	X	X	X	X
3	Quantitative tests - Stoll's dilution technique	X	X	X	X
4	Urine examination - Urine sedimentation test for nematodes (Stephanurus dentatus, Dictyophyma renale, Capillaria plica, etc)	X	X	X	X
5	Skin scrapping - 10% KOH digestion test	X	X	X	X
6	Blood parasite examination Direct smear: - Babesia - Theileria - Trypanosome - Anaplasma - Erhliachia - Hepatozoan spp Concentration & identification: Microfilaria identification from blood (Modified Knott's method) - Dirofilaria immitis - Dipetalonema reconditum Setaria species	X	X	X	X
7	Muscle parasites: -Pepsin digestion test		X	X	X
8	Faecal culture simple tube method culture tube method Baermanns technique Protozoa, large nematodes				X
9	Recovery of helminths: Parasitic identification of gastro-intestinal nematodes of sheep and cattle nematode larvae/metacercariae from herbage and enumeration of nematode larvae in herbage		X	X	X
10	Tick identification – stereo zoom technique			X	X
11	Prepucial Trichomoniasis test and identification of trichomonas				X
12	Worm staining & preservation			X	X
13	Immunological test -ELISA for Fasciola				X