

— MANUAL ON — **PARASITOLOGY**



by

**P. Chandrawathani, B. Premaalatha,
Jamnah Omar and Zaini Che Mamat**

Published by

JABATAN PERKHIDMATAN VETERINAR
KEMENTERIAN PERTANIAN & INDUSTRI ASASTANI MALAYSIA

DEPARTMENT OF VETERINARY SERVICE
MINISTRY OF AGRICULTURE AND AGRO-BASED INDUSTRY MALAYSIA



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FOREWORD

A message from the Director-General of Department of Veterinary Services, Malaysia



First of all, I would like to express my gratitude and appreciation to all who have been involved and contributed towards the preparation of this book, entitled “Manual of Parasitology”. This book is a useful guideline for the technical laboratory practices in the Department of Veterinary Services particularly in the Parasitology and Haematology divisions for the detection and identification of parasites in animals.

Over the past several years, the techniques given in this book have been shared with veterinarians, laboratory technicians and the industrial trainees who came to Veterinary Research Institute (VRI), Ipoh, Perak. Credit should be given to pioneer staff who have adopted, modified and refined various techniques to suit Malaysian conditions, reflecting their years of experience in this field.

Traditionally, the laboratory diagnosis of parasitic disease is fairly simple and straightforward. The equipment and facilities are usually readily available and not costly. Nevertheless, suggestions to improve or new techniques that have been developed are welcome for inclusion in this manual. It is also important that basic techniques in parasitology are clearly understood and practiced because the techniques applied are fundamental for the laboratory diagnosis to help diagnose and assist veterinarians in the field.

Therefore, we hope that this manual may continue to serve as a useful reference for the safe conduct of work in the laboratories.

Dato’ Dr. Quaza Nizamuddin bin Hassan Nizam

Director-General of Veterinary Services

Department of Veterinary Services

Malaysia



ACKNOWLEDGEMENTS

The field of parasitology and the diagnosis of veterinary related infections involves detailed laboratory techniques, so that accurate information is given to the attending veterinarian for follow-up treatments and management. Thus, it is with great pleasure that the authors have compiled this manual which is hoped to be useful for technical staff and students working in parasitology and haematology laboratories. This manual is the result of the dedicated and passionate collection of information and photographs over a span of 30 years. It is aimed to give diagnosticians in parasitology and haematology a first hand step-by-step view of how to conduct basic techniques required and come out with good diagnoses. It is well known that parasitic disease diagnosis and haematology are the first and fastest diagnostic workout for a disease condition. As such, the techniques mentioned here can be efficiently conducted by technical staff with the help of this manual.

This manual is the dedicated compilation by authors: Dr Chandrawathani and the staff, Mdm Premaalatha Bathmanaban, Mr Zaini Che Mamat and Mdm Jamnah Omar; with supporting contribution from Mr Adnan Musbah, Mdm Nurulaini Raimy, Dr Khadijah Saad, Dr Zawida Zahari, Dr Fazly Ann Zainalabidin, Mr Erwanas Asmar Ismail and Mdm Debbra Marcel; during their tenure at the Parasitology and Haematology Laboratory of the Veterinary Research Institute from 1990 to 2018. They were assisted by the industrial training students from University Selangor, Ms Shanmugapriya Permalsivam and Ms Renuka Kanniah.

The authors thank the Director-General, Department of Veterinary Services, Dato' Dr Quaza Nizamuddin Hassan Nizam, and the Director of Veterinary Research Institute, Dr Faizah Hanim Mohd Saied, for their enthusiasm and support in the compilation of this manual. Also, to the field staff who provided samples for diagnosis and research, and other staff of the Veterinary Research Institute who were involved directly or indirectly towards the preparation of this manual.

We encourage constant networking among all related parties towards improving the diagnostic techniques so that we may help farmers and livestock producers in improving productivity. For further information, kindly contact pro@dvs.gov.my. This manual can also be accessed through the DVS Research Article Library (RAL) at <http://research.dvs.gov.my>.

With sincere thanks from all authors.

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BLOOD PARASITES

SMEAR PREPARATION

Microscope glass slides

When making a preparation on a slide it is most important that the slides are ABSOLUTELY GREASE FREE AND CLEAN.

Cleaning new slides

1. Soak the slides overnight in saturated potassium dichromate in concentrated sulphuric acid.
2. Remove the slides from above solution and wash the slides thoroughly with water.
3. Wipe the slides with a clean towel.
4. Store the slides in a clean slide box.

A) Blood smear

Definition of smear

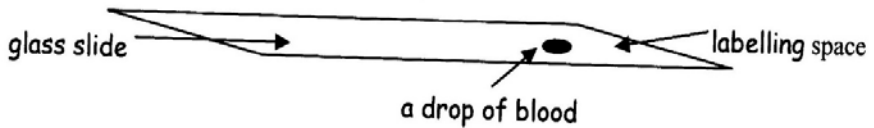
The body fluid is spread evenly onto one surface of a microscope glass slide. This preparation is known as a smear, sometimes called a film. Common fluids which are used to make smears are blood, intestinal contents, etc. (e.g. lymph, pus).

i) Thin smear:

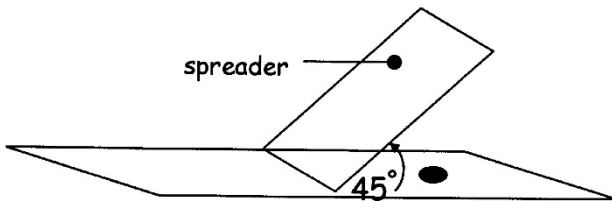
Small drops of blood for making smears may be taken from the ear vein of horses, cattle, sheep, goats, pigs, rabbits and dogs. When making smears from chicken, blood may be taken by snipping off a comb-tip with scissors or by cutting off the tip of the claw with scissors. For white mice, blood sampling is by snipping off the tip of the tail with scissors.

Procedures:

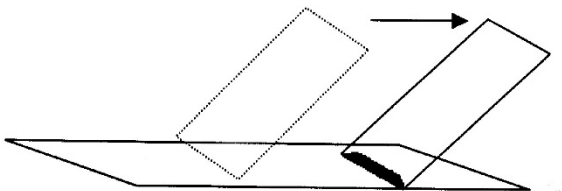
1. Place a very small drop of blood (pin-head size) at one end of a clean slide.



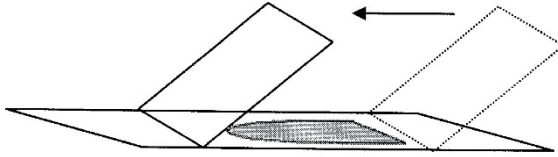
2. Hold the ends of the slide between the thumb and fore-finger.



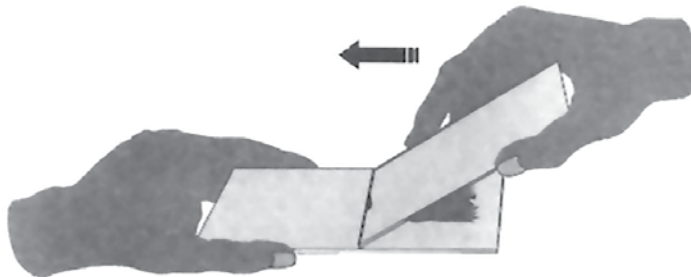
3. Apply the lower edge of the spreader to slide at an angle of about 45° and wait till the blood extends along the spreader edge, then keeping even contact.



4. Push the spreader forward along the slide. This action pulls the blood over the slide, leaving a thin smear.



5. Dry the film quickly by waving it vigorously in the air.
6. Rapid drying carried out in this way causes the blood cells to spread and show more surface for examination.



The picture shows how a proper smear is made.

ii) **Thick smear:**

Although most blood parasites may be detected without difficulty in thin blood smears during the acute stages of infection, parasites usually are not numerous in chronic infections. Thick blood smears are of value in chronic infections to quickly establish whether parasites are present in the blood. The thick film is opaque to transmitted light mainly because of the haemoglobin content. When the haemoglobin is removed, there is enough transparency for microscopic examination. Lysis with diffusion of the haemoglobin into the staining solution occurs while the film is being stained. Therefore, the film must not be fixed – that is to say, the blood proteins must not be coagulated by chemical or other means as is necessary for thin film. Lysis occurs readily enough unless the films are old when some degree of autofixation takes place. The drying of the blood films may take an hour or longer and the preparation should therefore be protected from flies and other insects during this time.

Procedures:

1. Place a drop of blood on a clean slide.
2. Spread the blood evenly with the corner of another slide. The correct thickness of the smear will be when the bands of a watch can be seen through it.
3. Keep the smear horizontal and protect it from dust and flies while it dries.

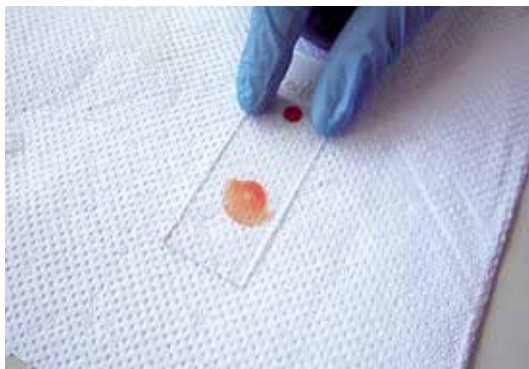
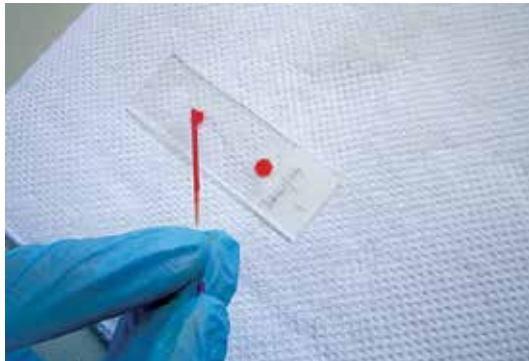


iii) **Wet Blood smear:**

Blood is not smeared onto the slide but is spread by pressure from the weight of the cover slip. It is used for detection of living trypanosomes and microfilaria.

Procedure:

1. Put a drop of blood on a clean slide.
2. Place a clean dry coverslip over the drop of blood.
3. Examine the film immediately under the microscope using 10× or 20× objective.



B) Blood tests available in Veterinary Research Institute (VRI)

Test	Test available in VRI	Test available in regional lab	Comments
Thin stained blood film	Yes	Yes	This is the most common method used for diagnosis, technical staff can identify organism quickly.
PCR	No	No	Done for research only and in collaboration with a university.
Wet blood film	Yes	Yes	Done in endemic cases, where quick diagnosis is needed, <i>in-situ</i> .
Thick stained blood film	No	No	Other techniques available
Hematocrit centrifuge	Yes	Yes	Commonly done
Dark ground	No	No	Lack of expertise / suitable equipment e.g. darkground microscope
Anion column	No	No	Lack of expertise / suitable equipment
Mice inoculation	Yes	No	Done only in VRI as there is lab animal facility. Strains are kept in liquid nitrogen.
<i>In vitro</i> culture	No	No	Lack of expertise / suitable equipment
IFAT	Yes	No	Done only in VRI, for research and screening purposes
ELISA	Yes	No	Done only in VRI, for research and screening purposes

C) Other techniques for parasite detection

i) Impression Organ Smear

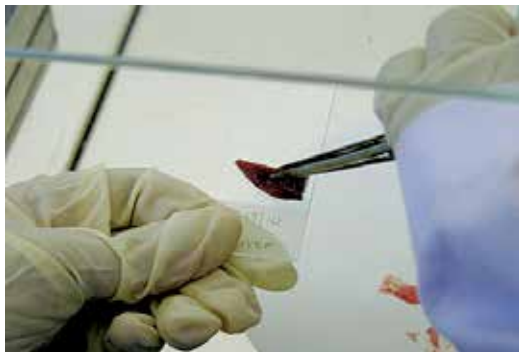
This is used for detection of blood protozoa in the organ and tissues of animals such as heart, spleen, liver, lung etc.

Procedures:

1. Cut a portion of the organ, e.g. heart, with a pair of scissors.



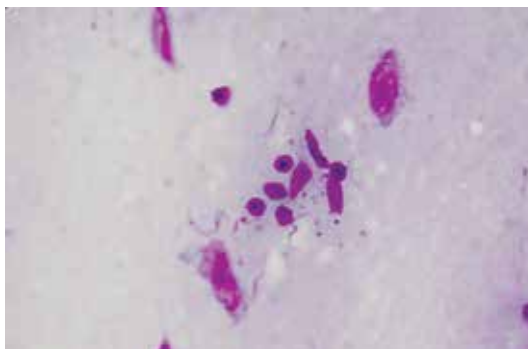
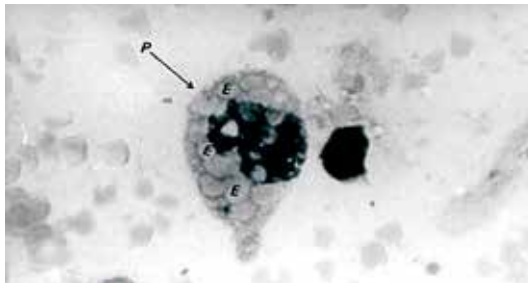
2. The tissue is gently pressed onto a clean slide



3. Dry the preparation in the air.



4. Stain the impression smear with a preferred staining reagent.
5. Example: a giemsa-stained liver impression smear of an infected experimental mouse showing positive *Trypanosoma* sp. (100× objective with 10× eye piece).



Note:

When dealing with organs, for example spleen which contains large amount of blood, the preparation may be blotted dry with filter paper.

ii) Brain Squash Smear

A large proportion of specimens submitted from cattle and buffaloes over the years only gave partial or negative results due to inadequacy of the range of specimens submitted. It is used for detection of blood protozoa in the organs and tissues of animals.

Procedures:

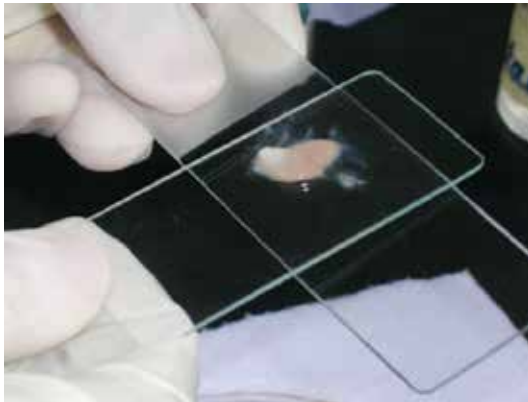
1. Remove brain from carcass.
2. Cut halfway down into brain-matter using knife, then pull apart forcibly using knife blade.



3. Put on the slide.



4. Place a pea-sized piece of brain material between two slides, squash and mash thoroughly.

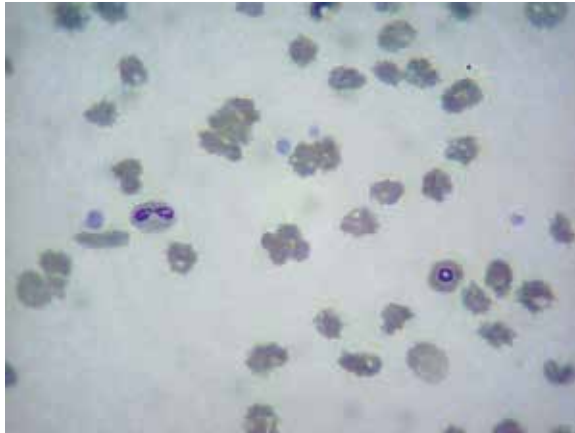


5. Wipe brain material off one slide using the small edge of a second slide.
6. Use the first slide to draw out brain matter on the second slide.
7. Fix brain smears in methyl alcohol after air drying.



8. Stain the smear with a preferred staining reagent.

9. Example of *Babesia* spp in a blood smear.



iii) **Intestinal Scrapping**

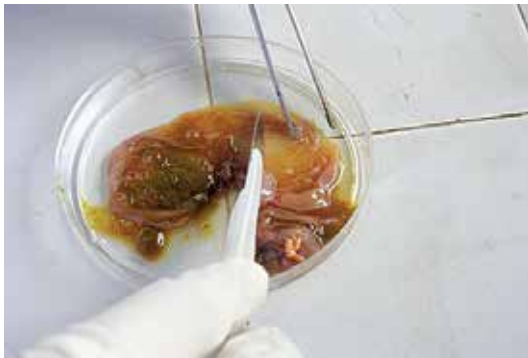
This technique is conducted for the detection of coccidian oocyst from intestine samples.

Procedures:

1. Cut open the intestine.

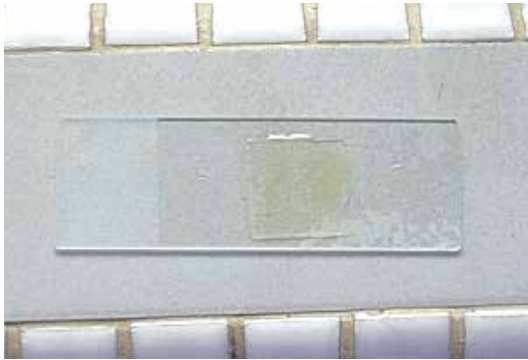


2. Scrape the intestinal lining with a scalpel.

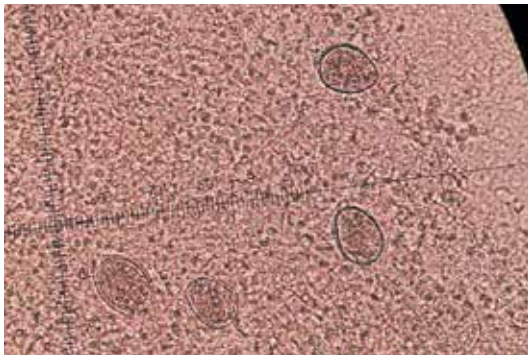


3. Put the contents of the scraping onto a slide and add a drop of water.

4. Put on the coverslip and examine under 10× objective of a compound microscope.



5. Example: Coccidian oocysts in a gut sample of goat.



FIXATION

Fixation of smears

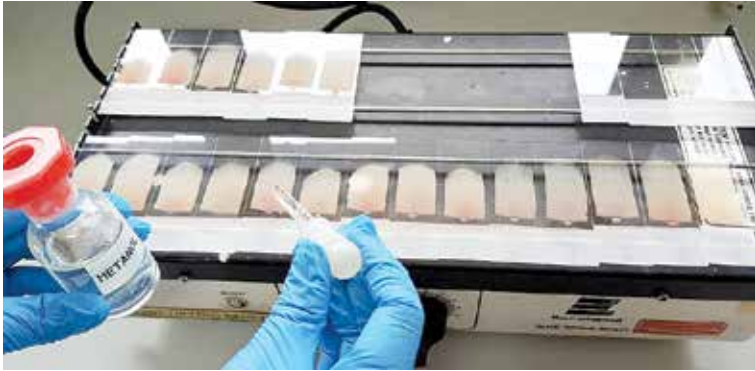
The materials for making smears would eventually deteriorate regardless of how well the preparation was made. Cells may shrink or stretch due to osmosis, or be digested by their own cellular enzyme. The materials may also be affected by bacteria or fungi such as moulds. Smears such as bacterial smears are usually fixed by passing the slide a few times through a flame, but this procedure is not practised in parasitology because the heat tends to break up red blood cells. Chemical fixation, usually with methanol is used to fix smears for 1-2 minutes followed by tipping-off the alcohol before staining.

Procedures:

1. Methanol is used to do the fixation.



2. The slide is air-dried before doing the fixation and followed by staining. A drop of methanol is placed on the slide to fix the smear.



STAINING

A) Leishman stain

Method:

Dissolve the stain with alcohol in a tightly stoppered bottle or a flask over a magnetic stirrer or with the help of glass beads. Keep the solution in an incubator at 37 °C overnight. At frequent intervals, agitate or shake it to mix the stain in the case using glass beads. Store in dark bottle. Filter the stain just before use.

(Please refer to Appendix for the recipes of Leishman stain.)

Staining procedures:

1. Cover the film with stain for ½ to 1 minute.
2. Add a double volume of buffer solution.
3. Mix by gently rocking or blowing.
4. Leave for 10 to 20 minutes.
5. Flush off stain and differentiate with buffer solution.
6. Drain and dry in air at room temperature.

B) Giemsa stain

Method:

Dissolve the stain (either powdered Giemsa or liquid Giemsa) with alcohol in a tightly stoppered bottle or a flask with a magnetic stirrer or with the help of glass beads. Keep the solution in an incubator at 37 °C overnight. At frequent intervals, agitate or shake it to mix the stain in the case using glass beads. Store in a dark bottle. Filter the stain just before use.

(Please refer to Appendix for the recipes of powdered Giemsa stain.)

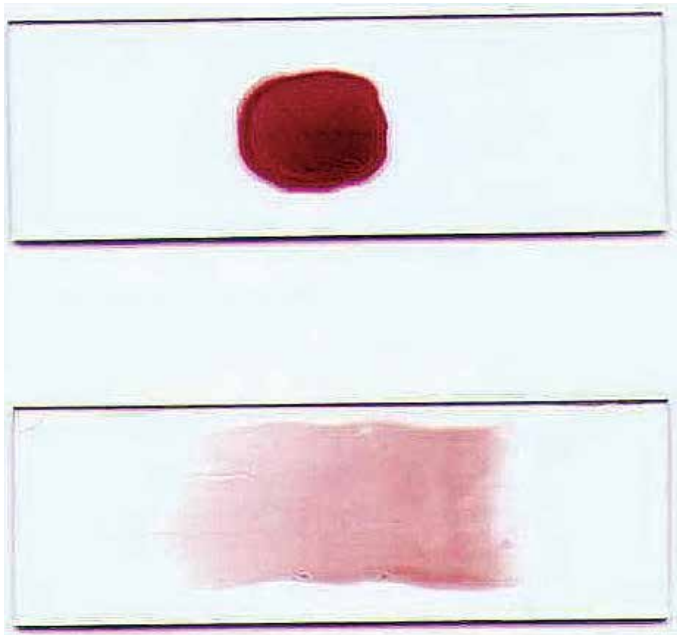
Staining procedures:

1. Place in 8% or 10% Giemsa solution, preferably with film facing downwards for ½ to 1 hour. (The best duration is 45 minutes.)
2. Flush off the stain with tap water.
3. Stand to dry.

C) Thick blood films for trypanosomes and microfilariae detection

Staining procedures:

1. Allow the thick film to dry thoroughly.
2. Stain the film facing downwards in 10% Giemsa, diluted with buffered distilled water pH 7.2 for 30 minutes.
3. Wash briefly in tap water.
4. Stand the slide to dry and mount.

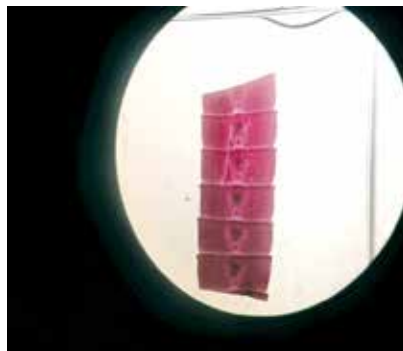


D) Aceto-alum-carmin staining

(Refer to Appendix for the recipes of aceto-alum-carmin stain)

Staining procedures:

1. Press the worm between two slides.
2. Fix overnight in formalin-acetic-alcohol or 10% formal saline.
3. Wash in running water for 1 to 8 hours.
4. Stain overnight in aceto-alum-carmin.
5. Wash off excess stain with distilled water until genitalia are seen as pinkish.
6. Wash well again in distilled water.
7. Dehydrate in ascending alcohol.
8. Clear in beechwood creosote or xylene and mount in Canada balsam or DePeX.



Picture shows worms in pinkish color.

(Note: For the recipes of phosphate buffer solution, please refer to Appendix.)

GASTRO- INTESTINAL TRACT (GIT) PARASITES

FAECAL EXAMINATION

INTRODUCTION

Fresh faeces is important for detection of helminth eggs/larvae as well as other parasites, such as oocyst of coccidian, which may be in the animal intestine in the process of leaving the animal with the intestinal excreta (Practical Parasitology, 1970). *(In the examination of poultry, faecal samples cannot be taken directly from the cloaca. If an assessment of individual birds is required, they should be caged separately and the fresh droppings collected. If individual bird assessment is not required then the fresh droppings can be collected from the house or pen.)*

The demonstration of eggs or larvae in faeces can indicate the presence of parasitic infection and facilitate the diagnosis of parasitic disease. Faecal material can be processed by:

1. Making a direct smear on a microscope slide for preliminary egg identification.
2. A flotation technique to concentrate eggs and separate them from debris for identification and counting.
3. Faecal culture to hatch the eggs and develop the larvae to the third stage for identification.

BASIC CONCEPTS OF FAECAL EXAMINATION

Collection of faeces

Faeces should be collected in a plastic bag/container from the rectum unless the animal is observed in the act of defaecation when the sample may be collected from the ground. Refrain from collecting faeces from the floor or ground as it may be old faeces which may have eggs already larvated.

Many of the methods used in faecal examination involve the separation of parasites, ova, larvae or cysts from the material with which they are mixed or in which they are contained, e.g. tissues, faeces, herbage, culture media.



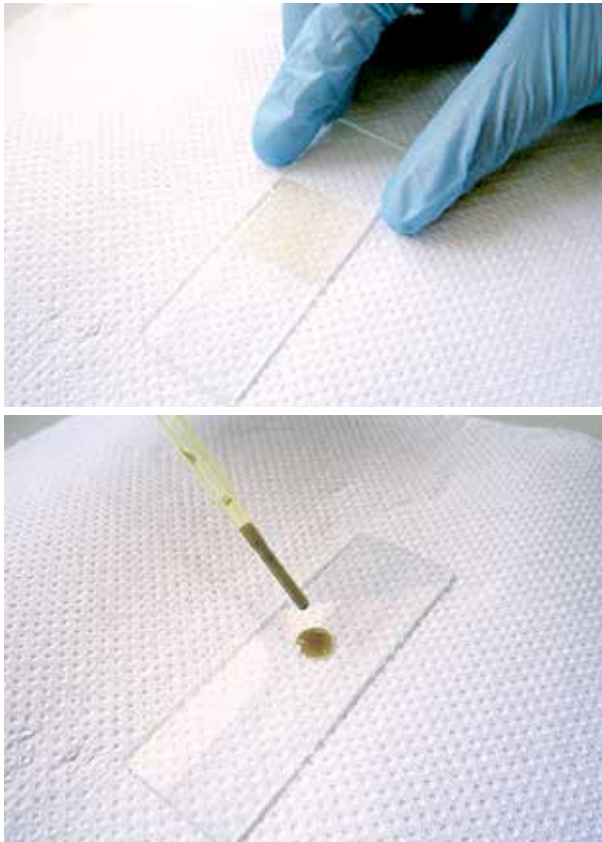
Fecal sample from Parasitology & Haematology Lab, VRI

Sampling techniques and submission

All samples should be collected fresh or directly from the animals. Fresh faeces can reveal some vegetative or trophozoite forms of protozoan parasites. Faeces must be labeled properly and sent to the laboratory within 24 hours, and kept refrigerated or preserved in 10% formalin when submission has to be postponed.

A) Simple or direct faecal smear (10 minutes)

1. Place a small amount of faeces on a microscope slide.
2. Add a drop of liquid to the faeces and mix thoroughly. The type of liquid added depends on the technique. In examining a liquid faecal sample for the presence of protozoan trophozoites (live active protozoa), saline is used if any extra liquid is needed. When looking for helminth eggs and protozoan cysts in a small sample (bird droppings, rectal smear etc), either water or iodine may be used.
3. Cover with a cover slip.
4. Examine the slide using the 10× objective, and then go over it with the 40× objective.

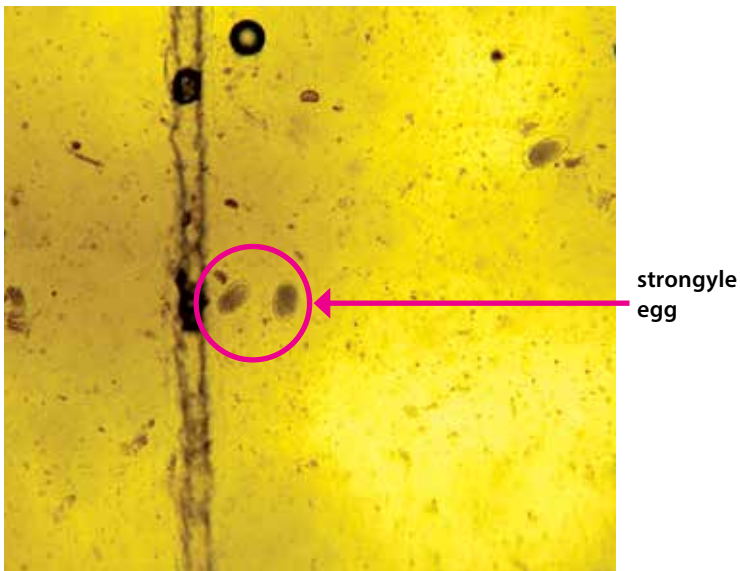


Advantages

A quick and easy method for detecting the presence of heavy worm infestation. Therefore, good for field work.

Disadvantages

1. Liable to overlook light infestations.
2. Qualitative only.
3. Frequently covered with detritus.



The picture shows the image of direct faecal smear under 10× microscope, positive for strongyle egg. (Picture of Parasitology & Haematology Section, VRI)

B) Simple flotation (15 minutes)

1. Emulsify 1 to 2 g faeces with saturated salt or sugar solution.

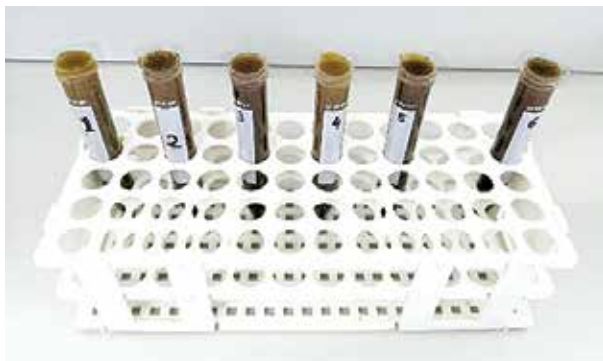


2. Filter through 85 mesh screen into a centrifuge tube.



3. Stand the tube erect in a rack.
4. Slowly 'top up' by means of a pipette with more saturated salt solution.
5. Lower a coverslip gently onto the meniscus at the mouth of the tube.

6. Allow to stand for 15 to 30 minutes.



7. Lift coverslip vertically up and put onto a slide.
8. Examine under 10x objective of a compound microscope for the presence of eggs.

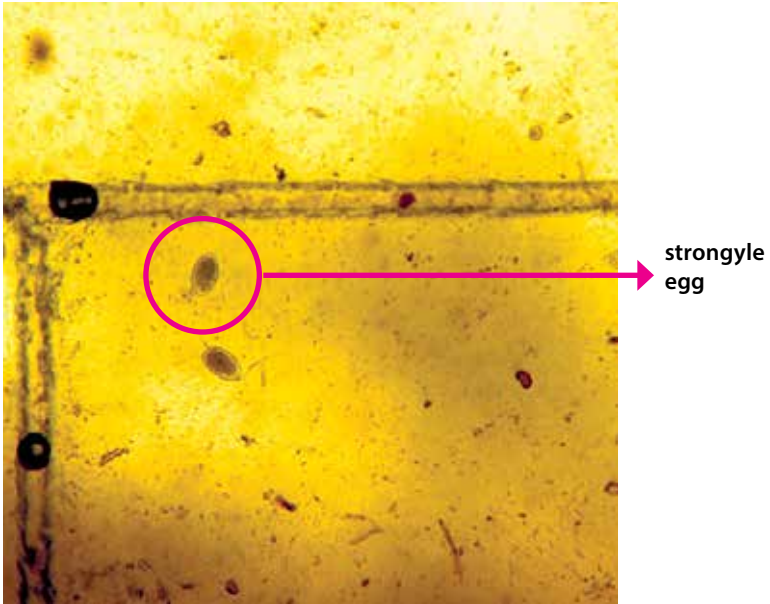
Advantages

1. Useful method for detecting presence of helminth ova even in light (helminth) infestation.
2. Eggs adhere to coverslip for easy examination.

Disadvantages

1. Method is qualitative.
2. For the detection of nematode eggs and coccidial oocysts only.

(Note: For quicker results, centrifuge tube with the coverslip on instead of standing for 15 to 30 minutes.)



The picture shows the image under 10× microscope.
(Positive for Strongyle egg.)

C) McMaster's method (flotation method)

(modified from *Manual of Veterinary Parasitological Laboratory Techniques*, 1986) (30 minutes)

1. Weigh 3 g of faeces into a jar.



2. Add saturated salt solution up to the 45 ml mark (1:15 dilution).



3. Mix contents by glass beads, automixer or pestle and mortar.



4. Filter through a 85 mesh screen and collect filtrate.



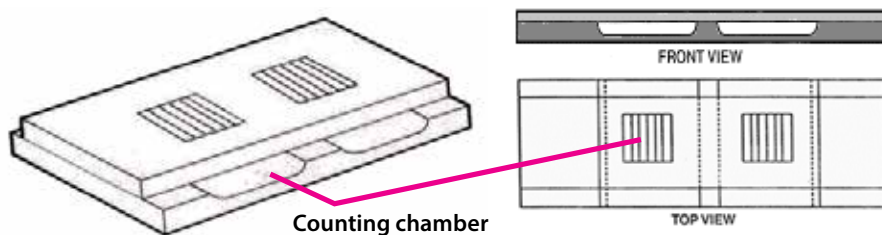
5. Mix the filtrate well and fill up both the counting chambers of a McMaster's slide.



6. Count all eggs seen within the ruled areas of **both** the chambers.



7. The mean of two counts is recommended in calculating egg counts.



Calculation:

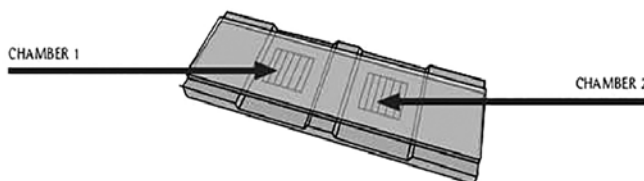
Volume of each counting chamber is **0.15 ml**.

0.15 ml of the solution of **3 g** of faeces in a volume of **45 ml** contain, for example x eggs.

$$\therefore \text{eggs in total volume (45 ml)} = \frac{x \times 45}{0.15}$$

$$\text{eggs in 1 g of faeces (e.p.g.)} = \frac{x \times 45}{0.15 \times 3}$$

\therefore Multiply the number of eggs counted by 100 to give eggs per gram (e.p.g.)



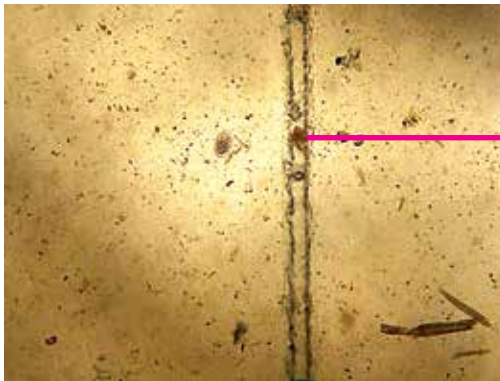
$$\text{Eggs / g of Faeces (EPG)} = \frac{(\text{Chamber A} + \text{Chamber B}) \times 100}{2}$$

Advantages

- Eggs float up for easy counting.
- No interfering faecal material.
- Fast and fairly accurate.

Disadvantages

- Liable to miss light infestation.
- Small quantity of faeces examined.
- Not good for all fluke eggs.
- Special slide required.



The picture shows the image of a McMaster slide with faecal sample under 10× microscope. (Positive)

Note : It is not often possible to identify strongyle eggs at genus level as the eggs of most strongylid and trichostrongylid species are similar in appearance and overlapping in size. If identification is necessary the faecal sample must be cultured to provide L3 larvae for further examination.

D) Sedimentation for detection of liver fluke eggs (1 hour)

1. Weigh 5 g of faeces into a bottle.
2. Add sufficient water and break up the faeces by an automixer, glass beads etc.



3. Filter through 85 mesh screen wire into a sedimentation or conical measuring cylinder (urine test glass).
4. Add water to wash the filter until the sedimentation cone is almost full.
5. Allow filtrate to sediment (10 to 15 minutes).
6. Pour or siphon off supernatant.
7. Wash deposit with fresh water and allow to sediment.
8. Repeat washing and siphoning till supernatant becomes clear.
9. When clear, wash deposit with water and make up to 10 ml in graduated centrifuge tube.

10. Pipette 2 ml at a time (depends on the amount of eggs present) onto a ruled petri dish and add 2 drops of methylene blue solution.



11. Examine under a stereo or dissecting microscope.



Calculation:

Report number of eggs seen as per gram of faeces.

(Note: For E. pancreaticum, use compound microscope to examine deposit.)

Advantages

- Can even detect a light fluke infestation.
- Larger amount of faeces used.

Disadvantage

- Not good for examination of strongyle eggs if examination is carried out under the stereo microscope.
- Not suitable for cattle below 6 months.



Picture shows the image of sedimentation deposit with methylene blue. (Positive)

E) Faecal culture (modified from Manual of Veterinary Parasitological Laboratory Techniques, 1986) [7 Days]

Purpose

To identify the third stage larvae of strongyle nematodes present in faeces.

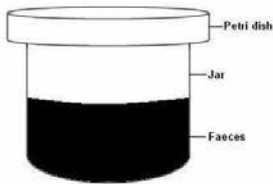
Principle

To provide suitable conditions for the hatching of eggs and larval development of the infective third stage (L3). The third stage of larvae can be recovered by the Baermann technique or alternative methods such as Roberts and O'Sullivan Technique and identified to the genus level (RVC/FAO Guide to Veterinary Diagnostic Parasitology).

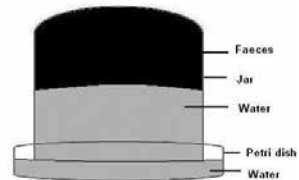
Method of faecal culture

1. The faeces is broken up by hand or by pestle and mortar.
2. The correct consistency of the faeces should be moist and crumbly but not wet.
3. This is done by the addition of water if the faeces is too dry and by drying sterile faeces if the specimen is too wet or diarrhoeic.
4. A culture jar is then filled with this mixture to about $\frac{1}{4}$ to $\frac{1}{2}$ mark.
5. The mixture is gently compressed by a flat bottomed tube.
6. The mouth and inside of the jar are wiped clean and the jar is then capped loosely.
7. The above is then left to incubate at room temperature for 7 to 10 days.
8. For harvesting of larvae (after 7 to 10 days), water is slowly added into the culture jar up to the brim.
9. A petri dish is placed over the mouth together with the jar, and then whole ensemble is turned upside down.

10. The moat between the rim of the jar and the sides of the petri dish is filled with a little amount of water (5 to 10 ml) and allowed to stand until the larvae have migrated out of the culture into the water (15 to 30 minutes).
11. The water containing the larvae is then collected from the petri dish into a centrifuge tube.
12. The centrifuge tube is allowed to sediment, the supernatant poured off, and the deposit is examined for larvae with a drop of Lugol's iodine.



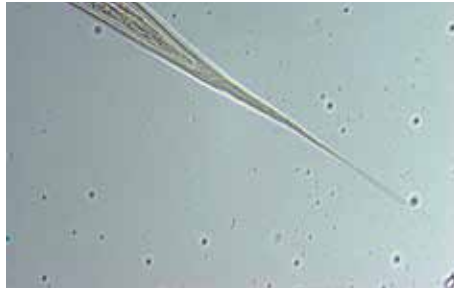
Faecal culture



**Harvesting of larvae from
the faecal culture**

Third stage larvae (L3) identification

1. Identification can be conducted with high accuracy by highly expert personnel. With the availability of keys and illustrations, one should be able to identify third stage larvae of nematodes.



Long Tail



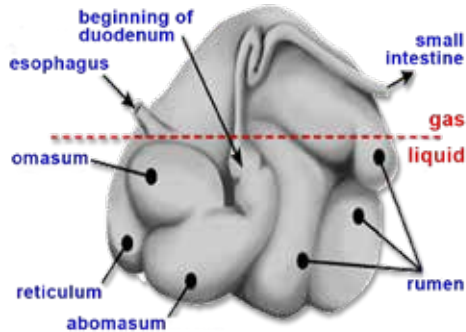
Medium Tail



Short Tail

F) Total worm count (TWC) for ruminants

This method is used for the examination of the stomach and intestinal contents of goat and sheep, but it may be adapted for the examination of the numbers of parasites in the gastro-intestinal tract of other animals.



Gastro-intestinal tracts (GITs)

1. The abomasum and small intestines are ligated separately and removed from the animal. They are then washed separately.



2. The stomach is cut open in a container and the contents collected. The stomach wall is washed thoroughly under a stream of water from a tap, the mucus membrane being carefully rubbed with fingers to remove any worms adhering to it. This washing is collected in the same container as the stomach contents.

3. The contents are now poured into a wide-mouthed bottle and allowed to sediment. The supernatant is poured away, and the contents made up to a volume of 1,000 ml with water (formalin may be added if preservation is required to give a final concentration of 10%).



4. The whole mixture is agitated vigorously and samples are removed with a wide-mouthed pipette. The vigorous stirring is continued throughout the process of sampling. The samples are transferred to a measuring cylinder until a total of 40 ml is collected.



5. Small quantities from the 40 ml sample are placed in a glass petri dish having ruled lines, diluted with water and the worms counted under a dissecting microscope. The total number of worms counted in the 40 ml sample is then multiplied by 25 to give the number of worms present in the stomach.



Calculation:

Let x be number of worms counted in 40 ml

$$\frac{x}{40} \times 1,000 = x \times 25$$

G) Collection and preservation of worms

i) Nematodes

1. Collect worms into a tube or petri dish containing 0.85% sodium chloride solution.
2. Shake gently to remove debris.
3. Transfer worms into hot 70% alcohol (80 °C). This fixes the worms in stretched position for easier identification.
4. Store in 70% alcohol containing 5% glycerine or helminth preservative fluid (refer to Appendix for formulae).
5. Clear the worms in lactophenol for the purpose of identification.

ii) Cestodes

1. Relax the worm in a water bath at 40 °C for about 15 to 30 minutes. This stretches (relaxes) the worm.
2. Drop it in a fixative e.g. 10% formalin or helminth preservative fluid. This will preserve the worm in its stretched position.

iii) Trematodes

1. Shake vigorously for 1 minute in 1% sodium chloride.
2. Add an equal quantity of saturated mercuric chloride solution and allow to stand for 10 minutes. This kills the worm in an out-stretched position.
3. Wash well in running water
4. Store in 70% alcohol, added with 5% glycerine or helminth preservative fluid.

H) Sporulation technique of cocci

The following steps are undertaken for sporulation of oocysts for coccidiosis species identification:

1. Fresh faecal samples are mixed and kept in 2.5% potassium dichromate.



2. The faecal mixture is broken down using a mortar and pestle.



3. The mixture is sieved and kept in a bottle which will be used as a stock bottle.



4. 7 ml of the sieved mixture is taken from the stock bottle and pipette into a 15 ml test tube.



5. The test tube is centrifuged at 2,000 rpm for 10 minutes.



6. 3 ml of saturated salt solution is added into the test tube.



7. The test tube is then centrifuged at 1,000 rpm for 10 minutes



8. Once centrifuged, the topmost whitish layer is removed and pipetted into another new 15 ml test tube.



9. In the new 15 ml test tube, distilled water is added to wash out the saturated salt solution.



10. The new 15 ml test tube is centrifuged at 3,000 rpm for 10 minutes. Step 9 and 10 is repeated one more time to wash off the salt solution.



11. Once the washing process is done, the supernatant is removed.



12. Finally, an equal amount of 2.5% potassium dichromate is added into the test tube with the sediment in it.

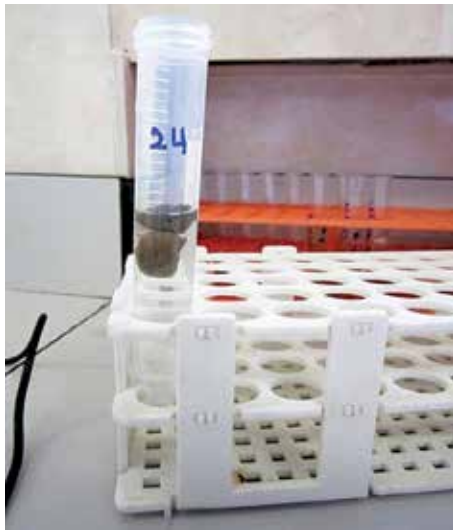


I) Further observation on the formal-ether concentration technique for faecal parasites

1. Transfer 7 ml of 10% formalin/distilled water to a 15 ml non-sterile centrifuge tube.



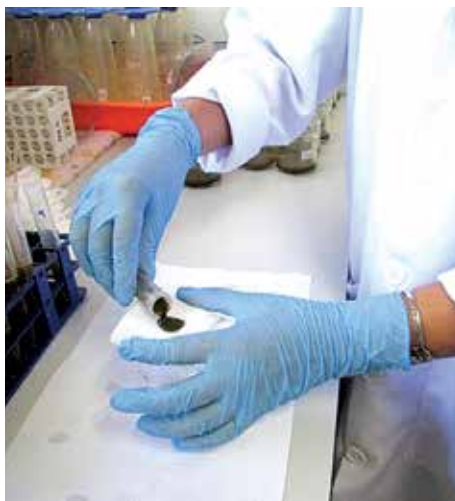
2. Take a specimen of faeces about the size of a pea with the end of a wooden stick.



3. Emulsify the faeces using the wooden stick in 7 ml of 10% formalin or distilled water in centrifuge tube.



4. Place a piece of gauze on a beaker and pour the whole content in the tube on the gauze and let the content pass through the gauze.



5. Clean the faecal debris in 15 ml centrifuge tube with pipe water and empty it.



6. Fill in 10% formalin solution/distilled water if the volume of filtrate is less than 7 ml.



7. Add 3 ml of diethyl ether into the filtrate, close the tube tightly and shake vigorously for 15 seconds.



8. Centrifuge the tube at 2,500 rpm for 5 minutes.



9. Pour off the centrifuged solution and leave the sediment at the bottom of tube.



10. Use a wooden stick to take out a bit of the sediment and smear on the slide.



11. Put a drop of iodine solution and cover it with a coverslip.



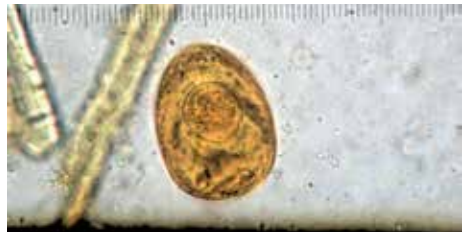
12. View under a compound microscope (10× and 40×).



Examples of egg found



Dicrocoelium



Moniezia



Schistosoma spindale



Paramphistomum

J) Techniques for *Blastocystis* sp. examination

(i) Preparing Jones medium

1. Add 960 ml distilled water into a beaker.
2. Add the chemical below:

Sodium chloride (NaCl)	-	7.087 g
Disodium hydrogen phosphate (Na_2HPO_4)	-	1.244 g
Potassium hydrogen phosphate (KH_2PO_4)	-	0.397 g
3. Dissolve the entire ingredient.
4. Discard 12.5 ml of the solution.
5. Add 1% yeast

Yeast	-	1 g
Distilled water	-	100 ml
6. Autoclave the solution.
7. Keep under 20 °C.

(ii) *In vitro* cultivation – Jones medium

METHOD

1. Inoculate a pea size amount of faecal sample into a sterile screw-top containing 3 ml of Jones medium supplemented with 10% heat-activated horse serum.
2. Incubate at 37 °C for 48 to 72 hours.
3. Observed under a light microscope at 400× magnification for the various forms of *Blastocystis* sp.
4. Consider negative after day 3 if none is present.
5. Smear the positive faecal culture on a slide.
6. Fix with methanol.
7. Let it dry.
8. Stain with 10% Giemsa for about 30 minutes.

A large teal-colored rounded square frame with a thin border and a slight drop shadow, centered on the page.

ECTO- PARASITES

ECTOPARASITES

SKIN SCRAPING: COLLECTION AND LABORATORY DIAGNOSIS

It is important to have adequate samples as mites may be absent from a small scraping from only one area of skin. The hair over the area should therefore be clipped short and discarded. The area selected should be the moist part on the edge of the lesion. Most mites will be on the periphery of active lesions. They will not usually be found in the thickened dried serous exudate.

In animals suspected of chorioptic or psoroptic mange, a sharp scalpel should be used with the blade held at an acute angle, shaving rather than scraping off the outer skin layer together with hair stumps. All specimens should be transferred or scraped directly into a small tube that can be securely stoppered. The usual samples received for examination is a skin scraping with some hair or wool.

Procedure:

1. Inspect under a low power of microscope. Mites may be visible.
2. Clip free and remove excess hair or wool.
3. Scrape some material onto a slide, mix a drop of 10% potassium hydroxide, warm and place a coverslip over the material.
4. Allow the preparation to clear for 5 to 10 min and then examine under medium and high power.
5. If no mite is seen, place the entire scraping (up to 5 g) in a boiling tube with about 10 ml of 10% potassium hydroxide.
6. Stand the tube in a beaker of water and gradually bring to the boil.

7. When all the crusts and hair have digested after 2 to 5 min in the water bath, allow the liquid in the tube to cool, then centrifuge to deposit the mites at 2,000 rpm for 2 min. A longer period will be required for smaller species. Avoid prolonged boiling in the caustic solution since the mites will eventually disintegrate.
8. Quickly decant the supernatant and then pipette the deposits off onto microscope slides for examination.
9. Make permanent mounts (e.g. for notifiable diseases such as sheep scab and parasitic mange of equines) by mixing the deposit with water, spinning down and decanting the dilute caustic solution, then adding 0.5 ml of glycerine jelly to the deposit.
10. Melt the jelly in a water bath, pour into a centrifuge and mix with the deposit by rolling the tube between the hands. With the mixture still warm, pour the fluid onto the slide and cover with a circular coverslip, 20 mm diameter. The jelly sets rapidly. Now examine the slide almost immediately under a low power microscope on a mechanical stage. Mark any mites or suspect mites, ring the mounts off and submit to the CVL for confirmation of the identification.
11. If no mites are seen, treat the wool or hair until stages 5 to 8. Demodex is easily made transparent by boiling in caustic solution and if present, should be detected in stages 1 to 4. Where samples from sheep are being examined, a flotation technique can also be used but only if there is an excessive amount of wool.
12. Immerse the wool in 20% potassium hydroxide in a test tube.
13. Incubate for 3 to 4 hours at 37 °C and centrifuge for 10 min at 2,000 rpm.
14. Tip off the supernatant.
15. Re-suspend in Sheather's sugar (454 g sucrose, 355 ml tap water, 6 ml 10% formalin).
16. Centrifuge for 10 min at 800 rpm and any mites present will float to the surface. Gently touch the surface of the sugar solution with the flat-ended glass rod and place the drop of fluid obtained onto a slide. Mount with a coverslip.



A) Techniques for preserving and mounting of ticks, lice and fleas

GENERAL

The correct method of preservation, mounting and labelling of insects is very important for proper identification and their usefulness for teaching purposes. Specimens of insects can be mounted in a chloral hydrate medium. Dehydration is not necessary before mounting in this medium. Small insects like lice can be mounted while alive. Some large specimens like ticks and fleas require macerating in a caustic solution before dehydration, clearing and mounting. All slides made with Berlese's fluid and other chloral hydrate media must be sealed after drying by ringing the coverslips with a waterproof substance such as Canada balsam, nail varnish, Glyceel or any other proprietary substances made for this purpose.

i) Simple rules in making a mounted slide

1. Use a coverslip of suitable size for the specimen.
2. Place an appropriate amount of mountant on the centre of the slide.
3. Arrange the specimen with head away and tail towards you except for fleas which are mounted lengthwise.
4. Only one specimen is to be mounted on a slide unless the male and female of the same species are required or a number of minute forms such as mites.
5. Affix the label with complete data on the specimen at the right side of the slide with the specimen in the correct viewing position i.e. upside down for a compound microscope and with head up for a stereoscopic microscope.

ii) **Method of macerating with caustic solution**

1. Puncture or make nicks in the body of the specimen to allow free penetration of solution.
2. Boil in 10% potassium or sodium hydroxide solution for about 5 minutes depending on specimens, or leave the specimen in this solution overnight.
3. Remove from caustic solution into water. Using a blunt instrument and with a gentle pressure, squeeze out liquefied contents of the abdomen.
4. Wash specimens well in distilled water containing a few drops of glacial acetic acid.
5. Transfer to fresh distilled water.
6. Dehydrate in ascending grades of alcohol e.g. 50%, 70%, 90%. Absolute 1 then Absolute 2 for 10 minutes in each one.
7. Clear in xylol and mount in DePeX, Canada balsam or any other suitable mountant.

(Note: Do not leave specimens too long in xylene as they get hardened quickly and mounting may be difficult.)

ACARINE (TICKS)

Preservation: 70% alcohol or 10% formalin

Note: Do not add glycerine in alcohol as this gives the specimens a shine. To preserve the natural colour of ticks, drop live specimens into a saturated solution of chloroform in 10% formalin. Transfer specimens to 70% alcohol after a month.

Mounting procedure: Follow method as described above.

MITES

Preservation: 70% alcohol with 5% glycerine added to prevent drying out of specimen in permanent storage.

Mounting: No maceration required. Mount directly in Berlese's fluid.

ANOPLURA (LICE)

Preservation: 70% alcohol

Mounting procedure: Follow method as described above

SIPHONAPTERA (FLEAS)

Preservation: 70% with 2% glycerine added to prevent drying out.

Mounting: Follow method as described above.

Note: For rapid clearing of specimens, lactophenol is recommended. After examination, specimens can be returned into the preservation.

B) Mounting procedures for *Culicoides*

1. Place the *Culicoides* in absolute alcohol for 30 minutes (dehydration).
2. Clear the *Culicoides* in phenol solution (phenol crystals dissolve in a small amount of absolute alcohol) overnight.
3. Remove the *Culicoides* from the phenol solution and place it in a drop of phenol solution on a microscope slide.
4. Examine the specimen under the dissecting microscope, detach the head and one wing from the specimen.
5. Place a coverslip over the whole specimen; place a small drop of Canada balsam at the edge of the coverslip so that it could seep into the specimen.

C) Mounting procedures for mites

1. Place the mites in lactophenol and leave it overnight.
2. Remove the mites from the lactophenol and rinse in water until the cloudy interface of lactophenol and water disappears.
3. Place a drop of Hoyer's medium or other suitable aqueous mountant in the centre of a clean microscope slide.
4. Place the mite in the mountant so that it is at the bottom of the droplet and arrange it on vertical axis, and place a coverslip over the specimen.
5. Ring the edge of the coverslip with a water-proof substance such as nail varnish (Cutex).



Trichodectes canis (Louse)



Ixodes sp. (Tick)

REFERENCES

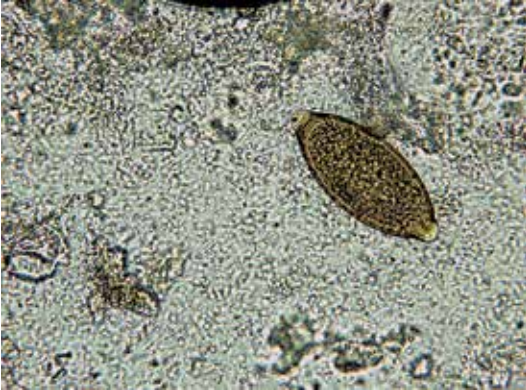
1. Chandler A.C. and Read C.P. (1975). *Introduction to parasitology* 10th edition. Wiley Eastern Private Limited, New Delhi. India. 16-17 pp.
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Appendix 1

COMMONLY FOUND EGGS

IN RUMINANTS

Description



Trichuris ovis

- Length 70 to 80 μm
- Width 30 to 42 μm
- Thick-walled
- Lemon-shaped with polar plugs
- Granular contents, no blastomeres



Toxocara vitulorum

- Length 69 to 95 μm
- Width 60 to 77 μm
- Sub globular
- Thick albuminous shell
- Granular contents
- Pitted surface to shell



***Moniezia* spp**

- i) *Moniezia expansa*
[mainly sheep]:
 - Size 50 to 60 μm
 - Triangular to pyramidal
- ii) *Moniezia benedeni*
[mainly cattle]:
 - Size 80 to 90 μm
 - quadrangular



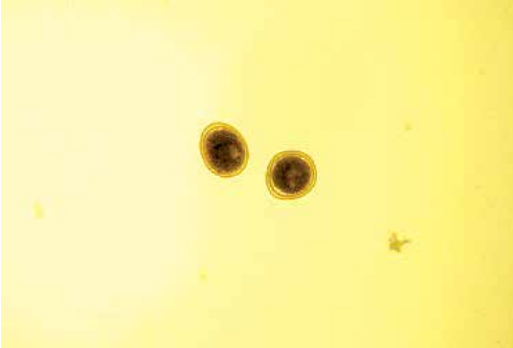
Strongyle

- Approximately 80 μm long
- Thin-shelled
- Broad ellipse
- Barrel-shaped side walls
- Blastomeres present, number vary

Appendix 2

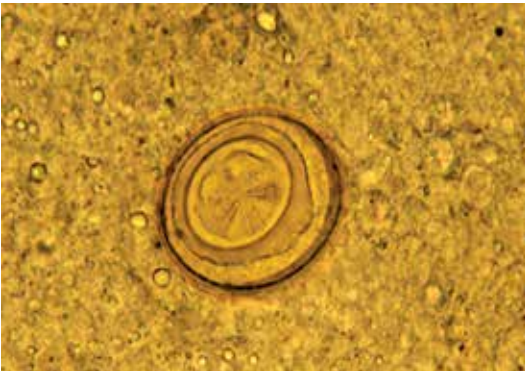
common parasites in faeces

Description of commonly found eggs in poultry



Ascaridia spp.

- Length 68 to 90 μm
- Width 40 to 50 μm
- Thick shell, smooth, three layers, slight barrel-shaped side-walls
- Contents unsegmented



Choanotaenia spp

- Size 45 to 55 μm
- The rostellum is ringed with about 18 slender hooks
- Possess a long distinctive filament



Heterakis spp.

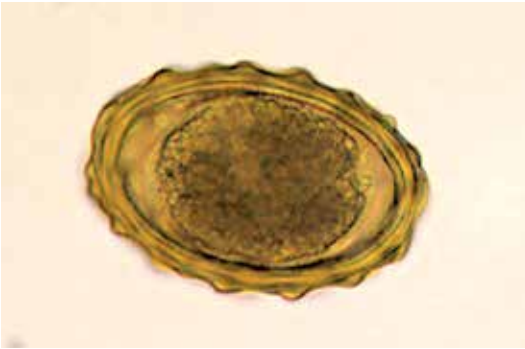
- Length 59 to 75 μm
- Width 31 to 48 μm
- Shell thick, smooth side-walls
- Contents unsegmented
- N.B. *Ascaridia* and *Heterakis* eggs are very similar and not easily distinguishable

Description of commonly found eggs in pigs



Trichuris suis

- Length 50 to 68 μm
- Width 21 to 31 μm
- Thick shell
- Two transparent, protruding polar plugs
- Unsegmented, brownish granular contents



Ascaris suum

- Length 50 to 70 μm
- Width 40 to 60 μm
- Thick walled, golden brown, thin inner yolk membrane, uneven lumpy albuminous outer wall
- Unsegmented, granular contents
- N.B. If only female worms are present, unfertilized eggs may occur: 88 to 90 $\mu\text{m} \times 44 \mu\text{m}$, albuminous layer of wall thin



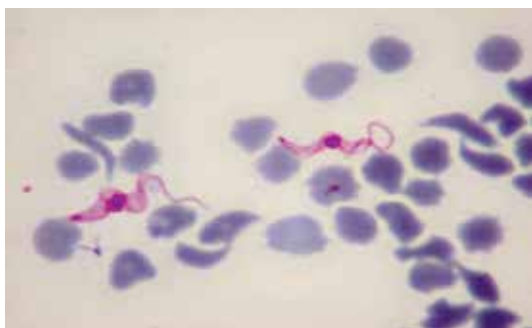
Strongyloides ransomi

- Length 40 to 55 μm
- Width 20 to 35 μm
- Single wall, greyish green
- Short, thick L1 larva present

Appendix 3

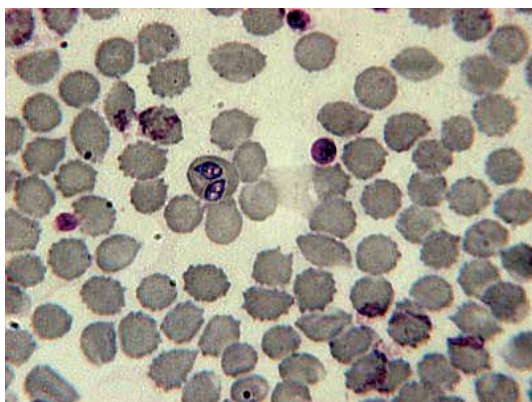
COMMON PARASITES FROM BLOOD

Description



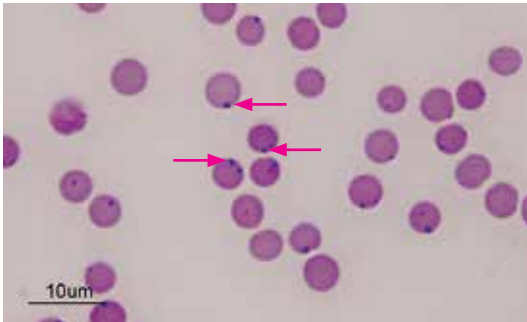
Trypanosoma evansi

- Causes Surra disease in animals
- Transmitted by tsetse flies
- Most important parasitic disease of camels and highly pathogenic for horses



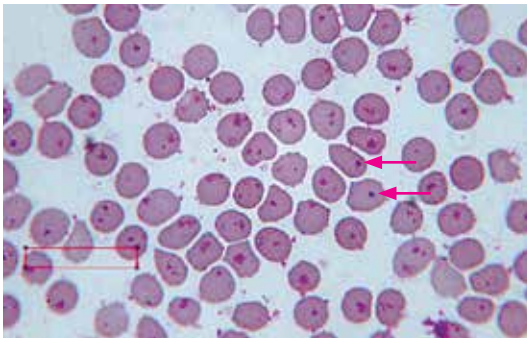
Babesia spp

- Causative agent for Babesiosis, a hemolytic disease
- Transmitted by biting insects
- Most commonly observed; *Babesia canis*, transmitted by *Rhipicephalus sanguineus* (tick)



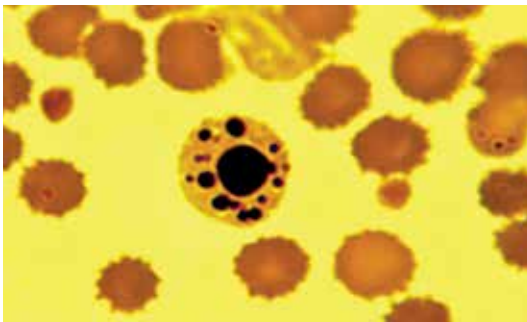
***Anaplasma* spp.**

- Causative agent for anaplasmosis
- Require intermediate tick hosts for maturation and flies as mechanical vector
- Causes haemolytic anaemia due to damage to red blood cells



***Eperythrozoon* spp.**

- *Mycoplasma suis* and *Mycoplasma parvum* are the common
- Uncultivated, wall-less bacteria of swine
- Parasitize the surface of erythrocytes and cause hemolytic anaemia in young pigs
- Transmission by common hog louse



Ehrlichia canis

- An obligate intercellular bacterium that acts as causative agent of Ehrlichiosis
- Transmitted by ticks *Rhipicephalus sanguineus*

Appendix 4

COMMON ECTOPARASITES



Mites



Demodex



Flea



***Tabanus* spp. (Biting fly)**



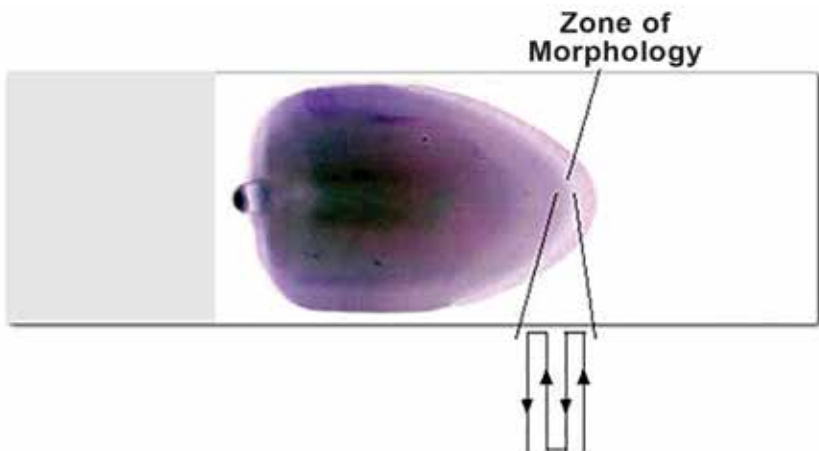
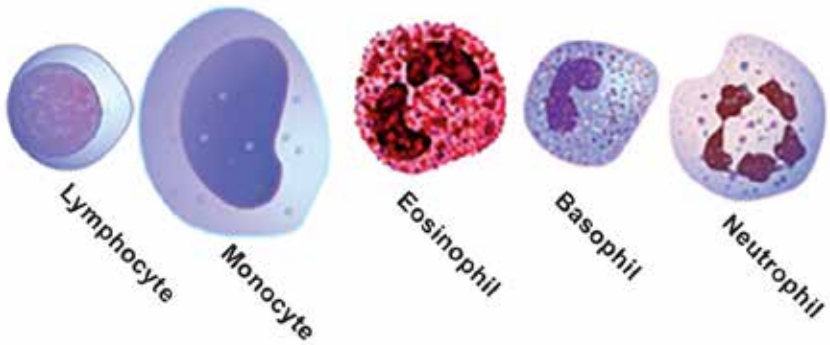
Tick

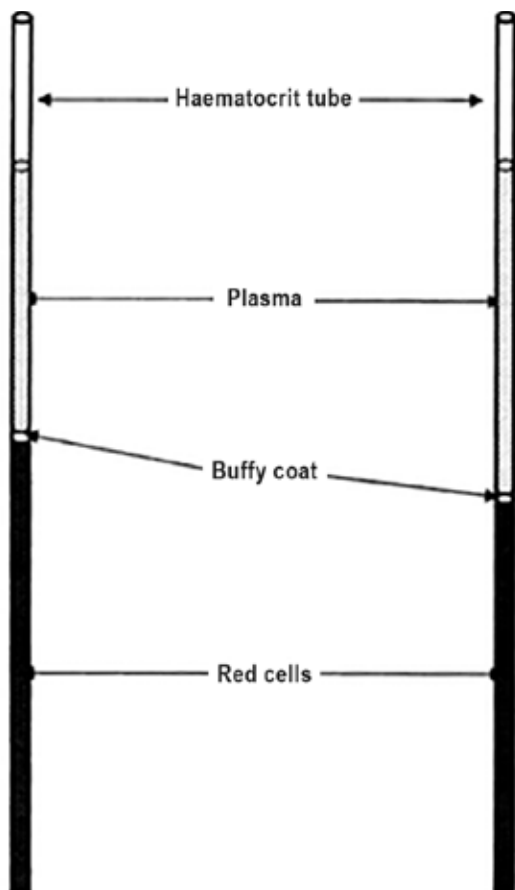


Lice

Appendix 5

WHITE BLOOD CELLS





Appendix 6

FORMULAS

1) Giemsa stain

Materials

Giemsa powdered stain	3.8 g
Methyl alcohol (Analar)	250 ml
Pure Glycerine (Analar)	250 ml

2) Phosphate buffer solution

For pH 6.8 (haematological examination)

Potassium dihydrogen phosphate (KH_2PO_4)	6.25 g
Disodium hydrogen phosphate (Na_2HPO_4)	5.0 g
Distilled water	1,000 ml

For pH 7.2 (blood protozoa)

Potassium dihydrogen phosphate (KH_2PO_4)	0.7 g
Disodium hydrogen phosphate (Na_2HPO_4)	1.0 g
Distilled water	1,000 ml

3) Preparation of aceto-alum-carmin stain

1. Boil excess carmine in a saturated solution of potassium alum.
2. Allow to cool and add glacial acetic acid to make up to 10%.
3. Stand for 24 hours and filter into dark bottle.

4) Mounting reagent preparation

Lactophenol Amann's

Phenol (absolute)	20 ml
Glacial acetic acid	20 ml
Glycerine	40 ml
Distilled water	20 ml

Berlese's Fluid

Distilled water	25 ml
Gum arabic	15 g
Chloral hydrate	100 g
Glycerine	10 ml
Chloral hydrate of cocaine	0.5 g



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