Fecal Culture and identification of infective third-stage larvae

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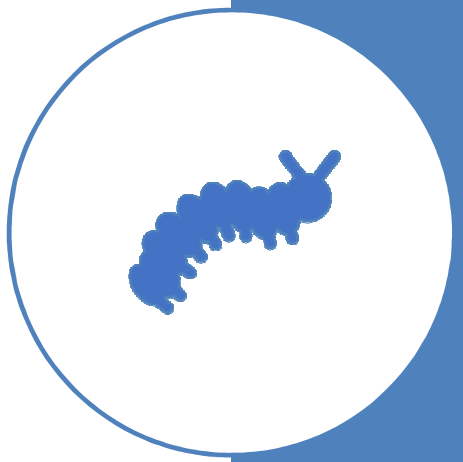
Culture and identification of infective third-stage larvae

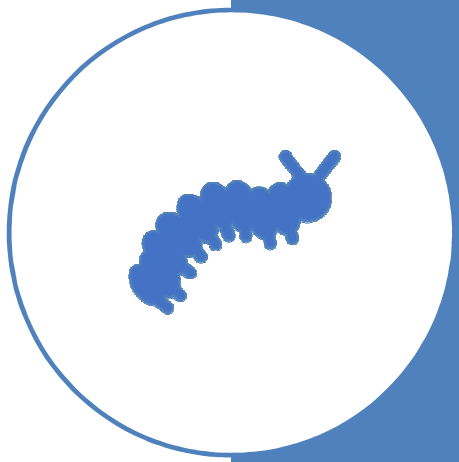
- The standard method for identifying eggs of trichostrongyle nematodes
- in feces is to culture the feces for 7–10 days and then collect the third stage (L3) larvae from the feces.
- The L3 can then be identified to genus, or in some cases to species.



How to do fecal culture?

- Feces are placed in a jar and Incubated at 25–27°C for 7–10 days.
- Keeping the culture moist by adding few drops of water is important.
- At the end of the incubation, the jar is filled with water and allowed to stand for 2–3 hours.

- The larvae will migrate into the water and the latter is poured into a cylinder for sedimentation.
- The larval suspension can be cleaned and concentrated by using the Baermann apparatus as described and then killed by adding a few drops of Lugol's iodine and examined microscopically.



Larval recovery

- Lungworm larvae can be recovered from fresh feces with the Baermann apparatus.
- This consists of a glass funnel held in a retort stand. A rubber tube, attached to the bottom of the funnel, is constricted by a clip.
- A sieve (aperture 250 µm), or a double layer of cheesecloth, is placed in the wide neck of the funnel, which has been partially filled with water, and a double layer of gauze is placed on top of the sieve.

- Feces are placed on the gauze and the funnel is slowly filled with water until the feces are immersed.
- The apparatus is left overnight at room temperature during which the larvae migrate out of the faeces and through the sieve to sediment in the neck of the funnel.
- The clip on the rubber is then removed and the water in the neck of the funnel collected in a small beaker for microscopic examination in a Petri dish.

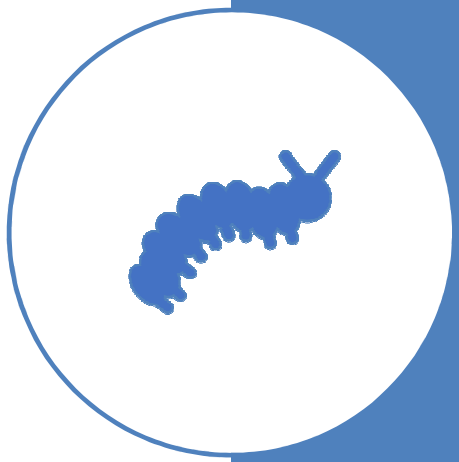
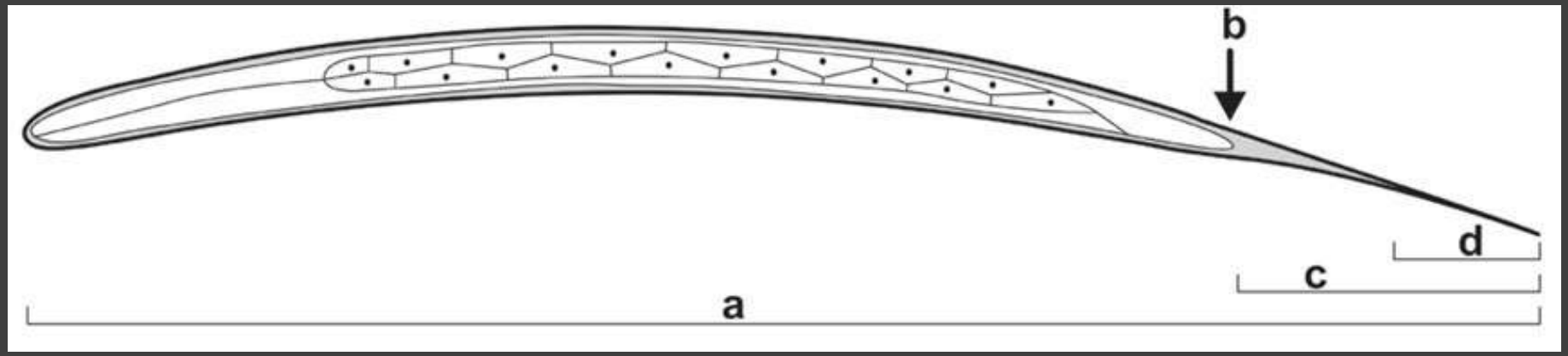


Fig. Baermann apparatus.

Larval identification.

- Identification the 3rd stage larvae of gastrointestinal nematodes is very important to differentiate between the different strongylida nematodes.
- Shape of the head, number of the intestinal cells and the tail sheath are the most important points on which the identification is resting.



- Source: Drawn from Borgsteede, F.H.M. & Hendriks, J., 1974, ‘Identification of infective larvae of gastrointestinal nematodes in cattle’, Tijdschrift Diergeneeskunde 99, 103–113.
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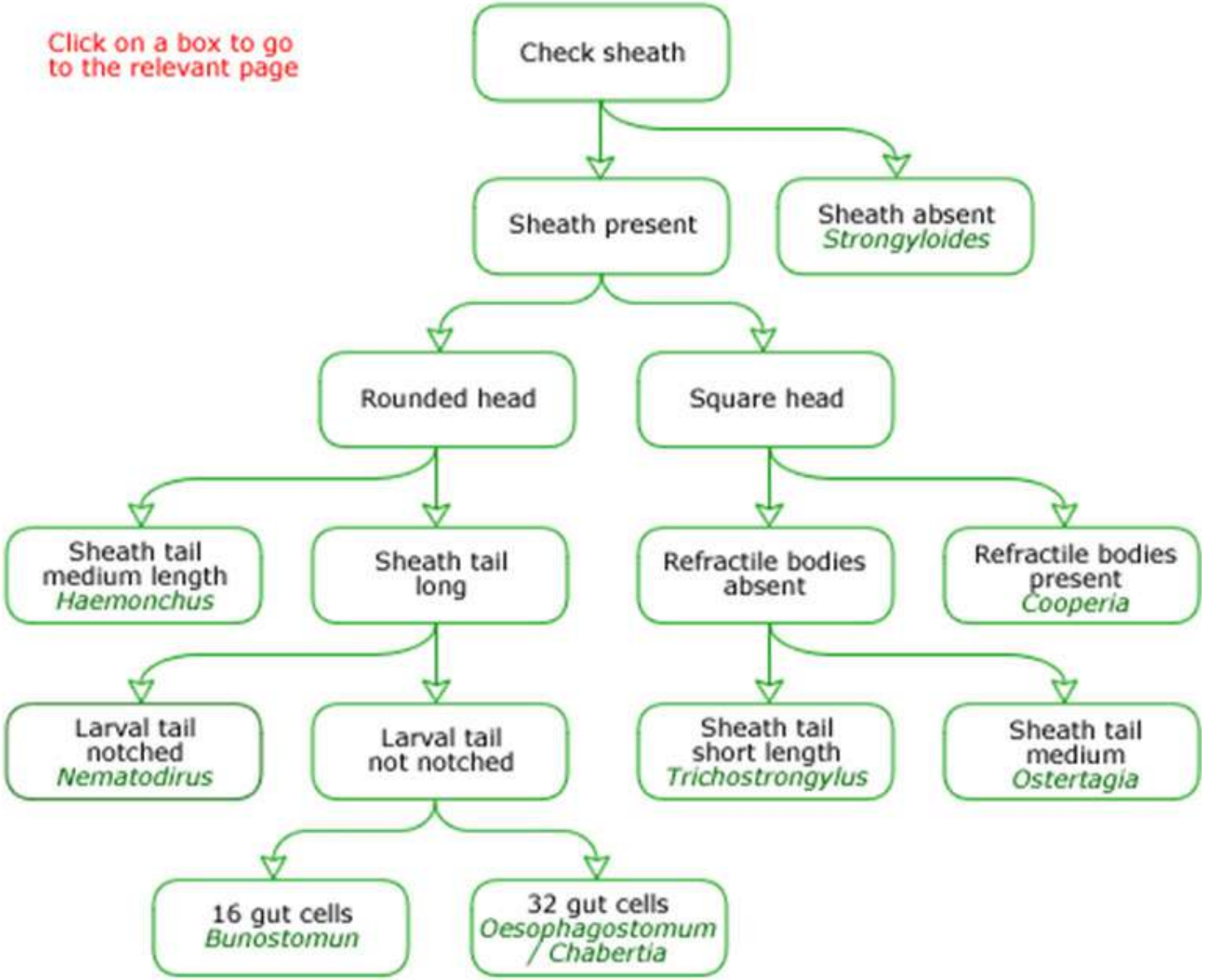
- Diagram of a nematode infective larva, depicting (a) total length, (b) tip of larva tail, (c) sheath tail extension and (d) filament.

Click on a box to go to the relevant page



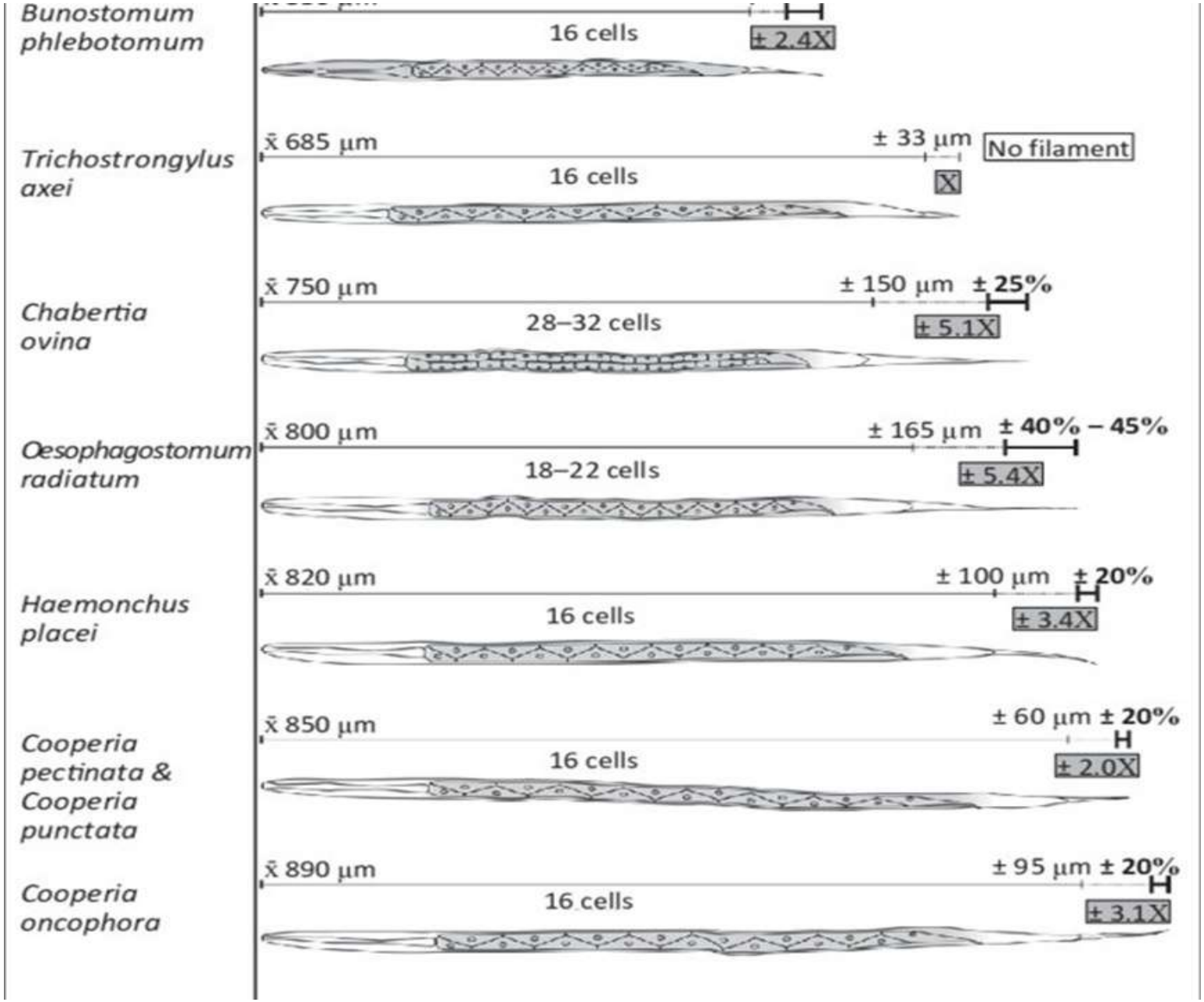

Bunostomum phlebotomum
16 cells
± 2.4X
Trichostrongylus axei
x̄ 685 µm
± 33 µm
No filament
16 cells
X
Chabertia ovina
x̄ 750 µm
± 150 µm
± 25%
28–32 cells
± 5.1X
Oesophagostomum radiatum
x̄ 800 µm
± 165 µm
± 40% – 45%
18–22 cells
± 5.4X
Haemonchus placei
x̄ 820 µm
± 100 µm
± 20%
16 cells
± 3.4X
Cooperia pectinata & Cooperia punctata
x̄ 850 µm
± 60 µm ± 20%
H
16 cells
± 2.0X
Cooperia oncophora
x̄ 890 µm
± 95 µm ± 20%
H
16 cells
± 3.1X

TABLE 1: Measurements of third-stage larvae of small ruminants, including sheath tail extensiona, 'X'-values and the proportion of the sheath tail extension comprising a filament.

Nematode	Length of STE (µm)		'X'-value of STE		Filament (% of STE)b
	Mean	Range	Mean	Range	
Trichostrongylus spp.c	30	18–32	1.0	0.6–1.1	Nil
Trichostrongylus falculatus and Trichostrongylus rugatus	51	46–56	1.7	-	Nil
Haemonchus contortus	74	65–82	2.5	2.2–2.7	10–15
Teladorsagia circumcincta	35	30–44	1.2	1.0–1.5	Nil
Cooperia spp. (mainly Cooperia curticei)	46	39–52	1.5	1.3–1.7	20–25
Cooperia oncophora	73	62–82	2.4	2.1–2.7	20
Cooperia spp. (from antelope)d	58	-	1.9	-	-
Oesophagostomum venulosum	168	122–207	5.6	4.1–6.9	-
Oesophagostomum columbianum	153	125–188	5.1	4.2–6.3	60–70
Chabertia ovina	123	101–150	4.1	3.4–5.0	25
Bunostomum trigonocephalum	99	85–115	3.3	2.8–3.8	40–50
Gaigeria pachyscelis	132	128–135	4.4	4.3–4.5	50
Nematodirus filicollis	261	-	8.7	-	50
Nematodirus spathiger	270	267–309	9.0	8.9–10.3	60
Nematodirus battus	171	-	5.7	-	-

STE, sheath tail extension.

a, For sources from which the values in the table were obtained, see Van Wyk, J.A., Cabaret, J. & Michael, L.M., 2004, 'Morphological identification of nematodes of small ruminants and cattle simplified', Veterinary Parasitology 119, 277–306. http://dx.doi.org/10.1016/j.vetpar.2003.11.012, PMid:15154594

b, For calculation of mean 'X'-values, the mean of Trichostrongylus spp. of sheep was used (Van Wyk et al. 2004 – see above).

c, Proportion of the STE that is filamentous (J. Van Wyk pers. obs., 1990, unless otherwise indicated in text).

d, Cooperia fuelleborni, Paracooperia serrata, Cooperioides antidorca (Mönnig, 1931).

TABLE 2: Measurements of third-stage larvae of cattle, including sheath tail extensiona, 'X'-values and the proportion of the sheath tail extension comprising a filament.

Nematode	**Length of STE (μm)**		**'X'-value of STEb**		**Filament (% of STE)c**
	Mean	**Range**	Mean	Range	
Trichostrongylus axei	32	25–41	1.1	0.8–1.4	0
Haemonchus placei	102	80–119	3.4	2.7–4.0	20
Ostertagia ostertagi	65	45–83	2.2	1.5–2.8	10
Cooperia pectinata / punctata	59	37–78	2.0	1.2–2.6	20
Cooperia oncophora	94	65–116	3.1	2.2–3.9	20
Bunostomum phlebotomum	73	58–96	2.4	1.9–3.2	50
Oesophagostomum radiatum	163	136–185	5.4	4.1–6.9	40–45
Nematodirus helvetianus	250	203–283	8.3	6.8–9.4	50
Nematodirus battus	165	-	5.2	-	-

STE, sheath tail extension.

a, For sources from which the values in the table were obtained, see Van Wyk, J.A., Cabaret, J. & Michael, L.M., 2004, 'Morphological identification of nematodes of small ruminants and cattle simplified', *Veterinary Parasitology* 119, 277–306. http://dx.doi.org/10.1016/j.vetpar.2003.11.012, PMid:15154594

b, For calculation of mean 'X'-values, the mean of *Trichostrongylus* spp. of sheep was used (Van Wyk *et al*. 2004 – see above).

c, Proportion of the STE that is filamentous (J. Van Wyk pers. obs., 1990, unless otherwise indicated in text)

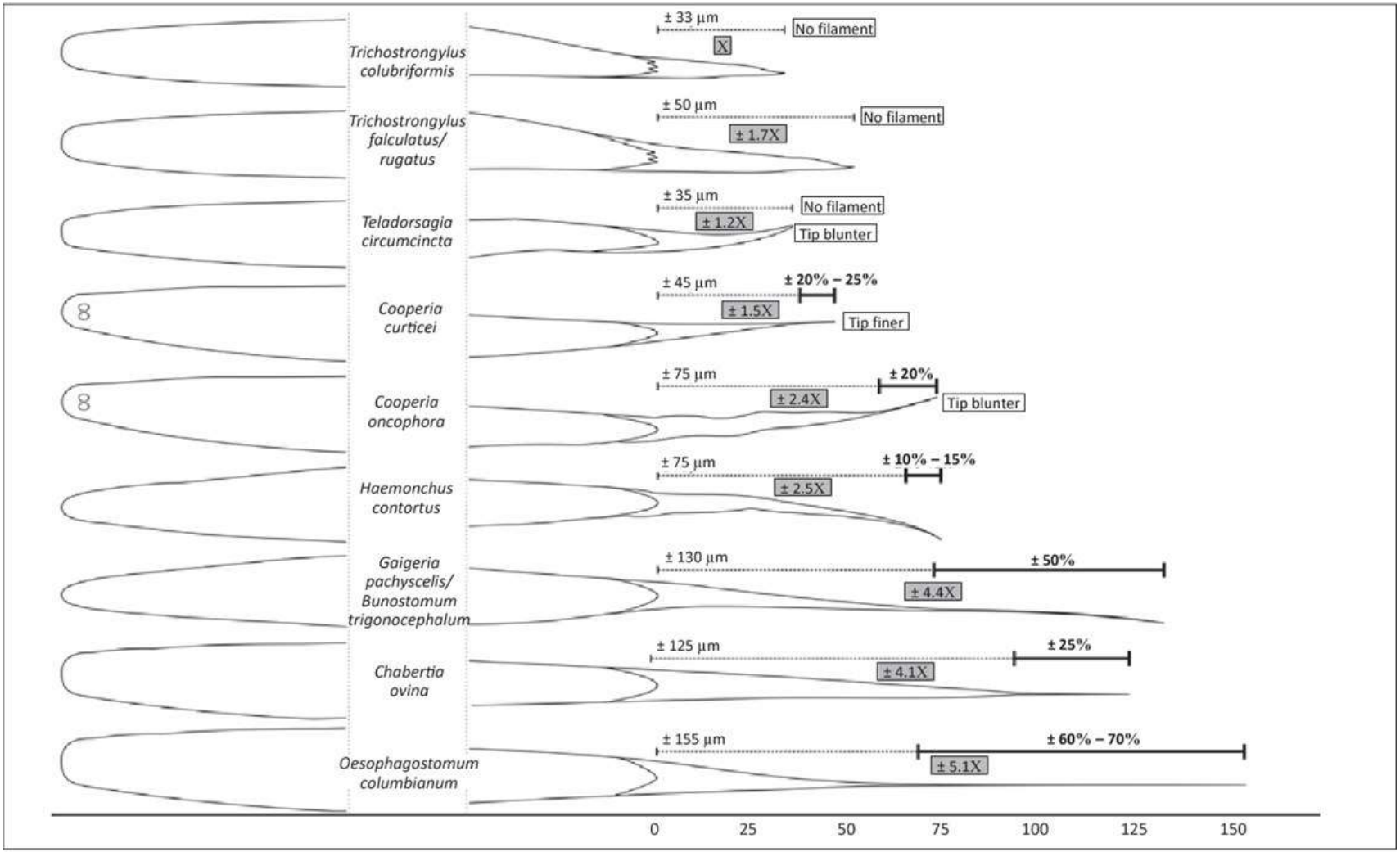
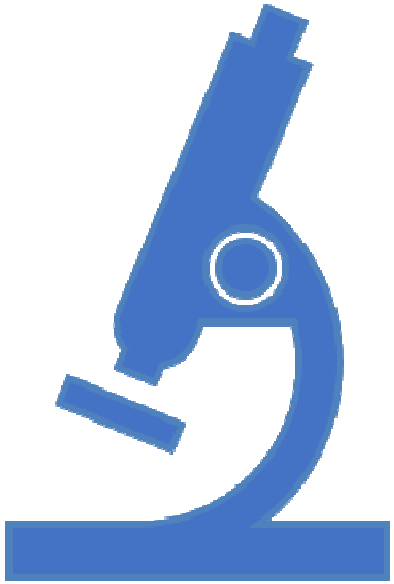


FIGURE 5: Cranial and caudal extremities of third-stage larvae of common nematodes of small ruminants, measured in micrometres (µm).



Fecal examination

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Fecal examination

- Although there is a progress in the use of serology and molecular methods in the diagnosis of pathogens; fecal examination for detection the presence of worm eggs or larvae considers the most common routine diagnostic tool.

Collection of Fecal samples

- Fresh fecal samples from large animals should be collected from the rectum and examined quickly as soon as possible. If rectal samples are difficult to be obtained, freshly deposited feces can be collected from the floor.

In case of large animals and small ruminants as sheep and goats,

- individual fecal samples are required, and a minimum of 10 samples per flock should be examined. A minimum amount of 5 gm feces should be collected, since this amount is required for carrying the different examination techniques.

In dogs and cats

- fresh feces can be collected from the cages in which they are housed. While in poultry, representative samples should be collected from different areas of the building. The feces should be stored in the refrigerator unless examination is carried out soon to stop hatching and development of the eggs and larvae.

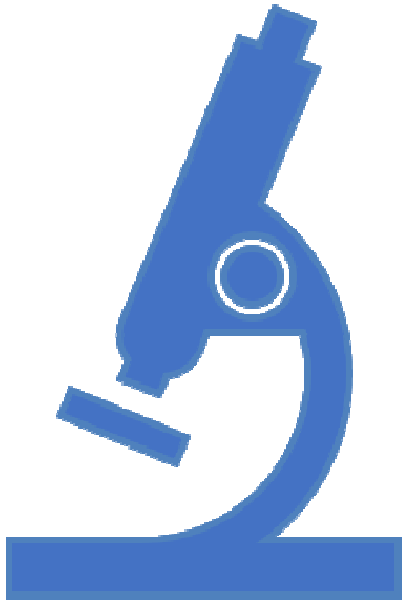
Methods of examination

Direct smear method

- On a microscope slide, few drops of water are mixed with equal amount of feces. Rolling the slide to allow the lighter eggs to leave the heavy particles. A coverslip is placed over the slide then examined under light microscope with X4 or X10. Due to the small amount of feces, this method is not suitable for the light infection

Flotation methods

- Flotation method is resting on when worm eggs are suspended in a solution with a higher specific gravity, the eggs or cysts will float to the surface. Because nematode, cestode eggs and protozoa cysts are small, so they float in a liquid with a specific gravity of 1.10–1.20.



Trematode eggs

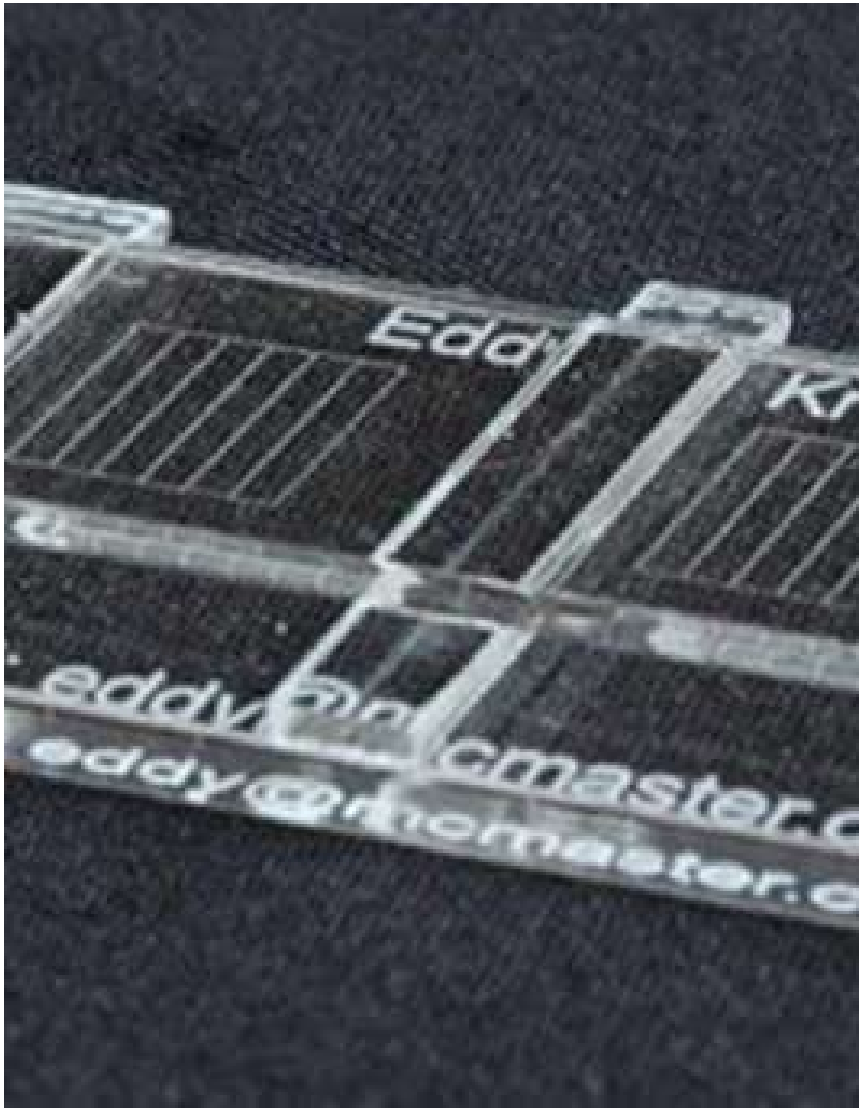
- are much heavier, so they require a specific gravity of 1.30–1.35. A saturated solution of sodium chloride (NaCl) or magnesium sulphate ($MgSO_4$) and sugar solution is prepared and stored for usage. Meanwhile, for trematode eggs, saturated solutions of zinc chloride ($ZnCl_2$) or zinc sulphate ($ZnSO_4$) are commonly used.

Direct flotation

- Approximately 2 gm of fresh feces are added to 10 mL of the flotation solution and,
- following thorough mixing, the suspension is poured into a conical plastic tube and more flotation solution added to fill the tube to the top.
- A coverslip is then placed on the surface of the liquid and the tube and coverslip are left standing for 10–15 minutes.
- The coverslip is then removed vertically and placed on a slide and examined under the microscope. The flotation of the eggs may be accelerated by centrifugation.

McMaster method

- This quantitative technique is used to estimate the number of eggs per gram of feces. It is important to know number of eggs per gram in case of gastrointestinal nematode infections as *Haemonchus*.



McMaster technique

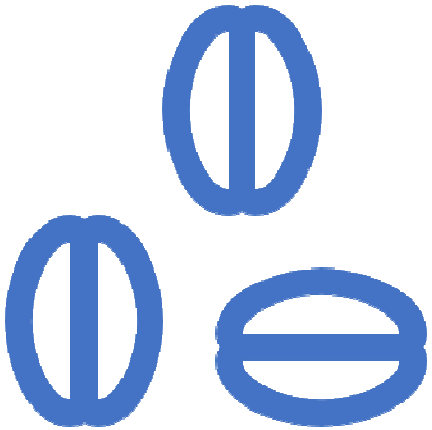
- 1 Weigh 3.0 g of feces.
- 2 Break up thoroughly in 42 mL of water in a plastic container.
- This can be done using a homogeniser if available or in a stoppered
- bottle containing glass beads.
- 3 Pour through a fine mesh sieve (aperture 150 µm, or 100 to 1 inch).
- 4 Collect filtrates, agitate and fill a 15-mL test tube.
- 5 Centrifuge at 1500 rpm for 2 minutes.
- 6 Pour off supernatant, agitate sediment and fill tube to previous level with flotation solution.

- 7 Invert tube six times and remove fluid with pipette to fill both
- chambers of a McMaster slide (Fig. 4.1). Leave no fluid in the
- pipette or else pipette rapidly, since the eggs will rise quickly in
- the flotation fluid.
- 8 Examine one chamber and multiply the number of eggs or larvae
- under one grid by 100, or two chambers and multiply by 50,
- to arrive at the number of eggs per gram (epg) of faeces:
- If 3 g of feces are dissolved in 42 mL
- Total volume is 45 mL
- Therefore 1 g is 15 mL
- The volume under the grid is 0.15 mL
- Therefore, the number of eggs is multiplied by 100. If two grids
- are examined, multiply by 50.

Zinc sulphate flotation method for counting fluke eggs

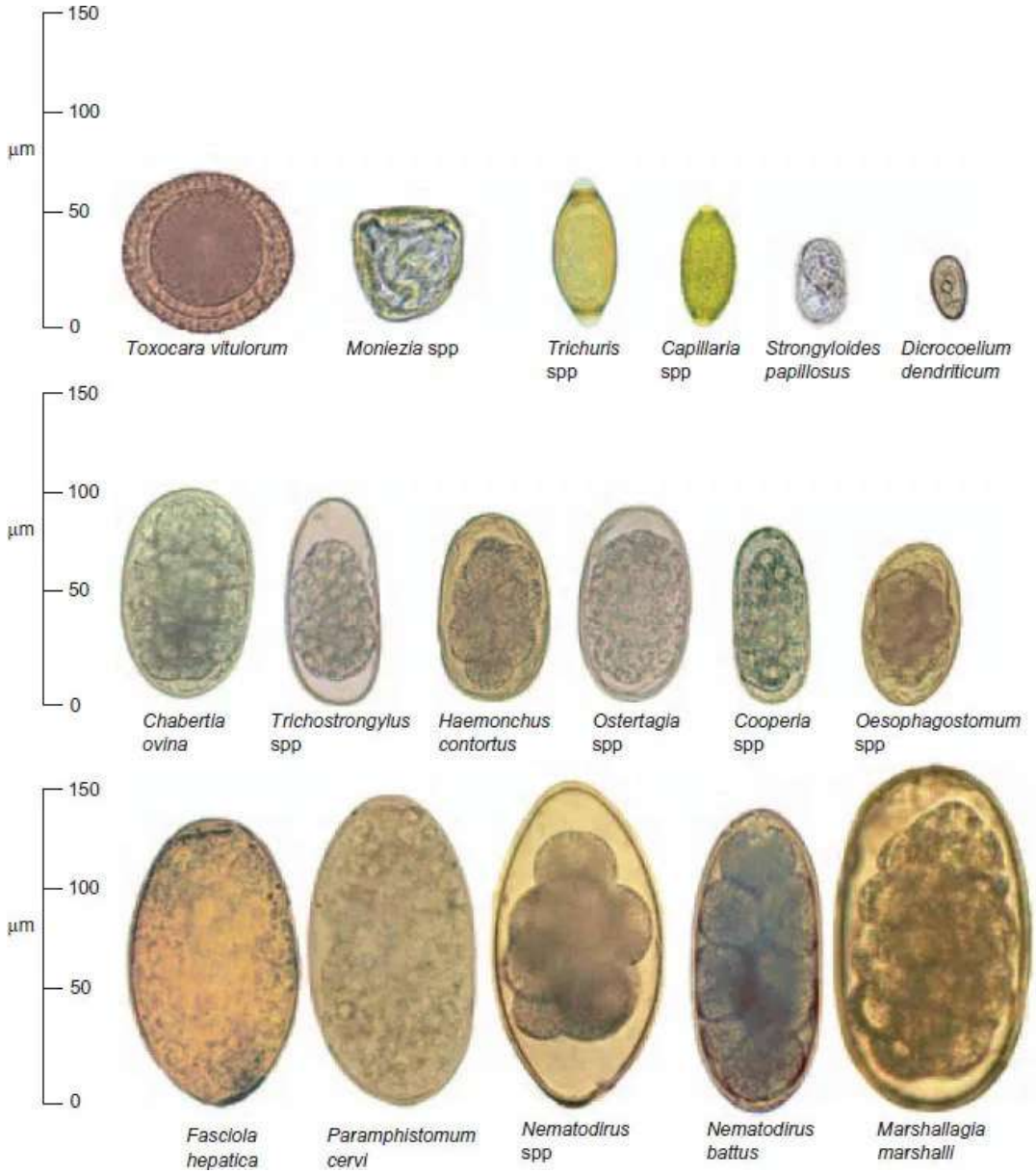
- Liver fluke eggs will not float in saturated NaCl solution but will float in saturated $ZnSO_4$ solution, which has a higher specific gravity. The procedure is exactly the same as that described for the improved modified McMaster method, with $ZnSO_4$ replacing NaCl.

Fecal sedimentation method for fluke eggs



- 1 Homogenise 3 g (sheep, goats) or 6 g (cattle) faeces with 50–60 mL of water in a beaker. Agitate or mix the faeces for about 30 seconds until broken down.
- 2 Sensitivity can be improved by increasing the amount of faeces examined.
- 3 Add 2 drops of Teepol detergent to the homogenate to improve the release of eggs from faecal material.
- 4 Pour through a strainer and collect filtrate into beaker or collecting bowl.
- 5 Strain filtrate through a second screen (~250 µm aperture) into a conical flask.
- Half fill the beaker with water and wash through the screen into the conical flask.

- 7 Allow filtrate in the conical flask to sediment for 3 minutes.
- 8 Siphon off supernatant with a suction pump, or manually with a large pipette, taking care not to disturb the sediment.
- 9 Step 7 may be repeated by adding more water to the sediment to further clean the sample.
- 10 Differentiation of the eggs can be enhanced by adding a couple of drops of methylene blue to the final sediment.
- 11 The sediment can either be examined in a Petri dish using a dissecting microscope or under a compound microscope by pipetting a small volume on to an ordinary microscope slide with a long coverslip (40 × 22 mm) and repeating until all the sediment has been examined.
- 12 Scan the Petri dish, or slides, systematically for the presence of fluke eggs.


150
100
µm
50
0
Toxocara vitulorum
Moniezia spp
Trichuris spp
Capillaria spp
Strongyloides papillosus
Dicrocoelium dendriticum
150
100
µm
50
0
Chabertia ovina
Trichostrongylus spp
Haemonchus contortus
Ostertagia spp
Cooperia spp
Oesophagostomum spp
150
100
µm
50
0
Fasciola hepatica
Paramphistomum cervi
Nematodirus spp
Nematodirus battus
Marshallagia marshalli

INTERNAL PARASITES OF DOGS & CATS

MAJOR CANINE PARASITES

ASCARIDS

Toxocara canis

Toxascaris leonina

HOOKWORMS

Ancylostomum caninum

Uncinaria stenocephala

WHIPWORM

Trichuris vulpis

TAPEWORMS

Dipylidium caninum

Taenia pisiformis

PROTOZOA

Isospora canis

Isospora ohioensis

Giardia canis cyst

Giardia canis trophozoite

Otodectes cyanotis

Demodex cati

MAJOR FELINE PARASITES

ASCARIDS

Toxocara cati

Toxascaris leonina

HOOKWORMS

Ancylostomum tubaeforme

Uncinaria stenocephala

TAPEWORMS

Dipylidium caninum

Taenia taeniaeformis

PROTOZOA

Isospora felis

Isospora rivolta

Giardia felis cyst

Giardia felis trophozoite

Cryptosporidium felis

Toxoplasma gondii

Otodectes cyanotis

Demodex cati

MINOR CANINE PARASITES

Physaloptera rara

Neospora caninum

Sarcocystis

Paragonimus kellicotti

Eucoleus aerophila

Eucoleus böhmi

Baylisascaris procyonis

Cryptosporidium canis

Ascaris canis

Macracanthorhynchus hirudinaceus

Spirocerca lupi

Spirometra mansonoides

Diphyllobothrium

Nanophyetus salmincola

Heterobilharzia americanum

Balantidium coli

MINOR FELINE PARASITES

Eucoleus aerophila

Paragonimus kellicotti

Nanophyetus salmincola

Spirometra mansonoides

Diphyllobothrium

Platynosomum

Ascaris

Aonchotheca putorii

Sarcocystis

Physaloptera rara

Panacur

Interval

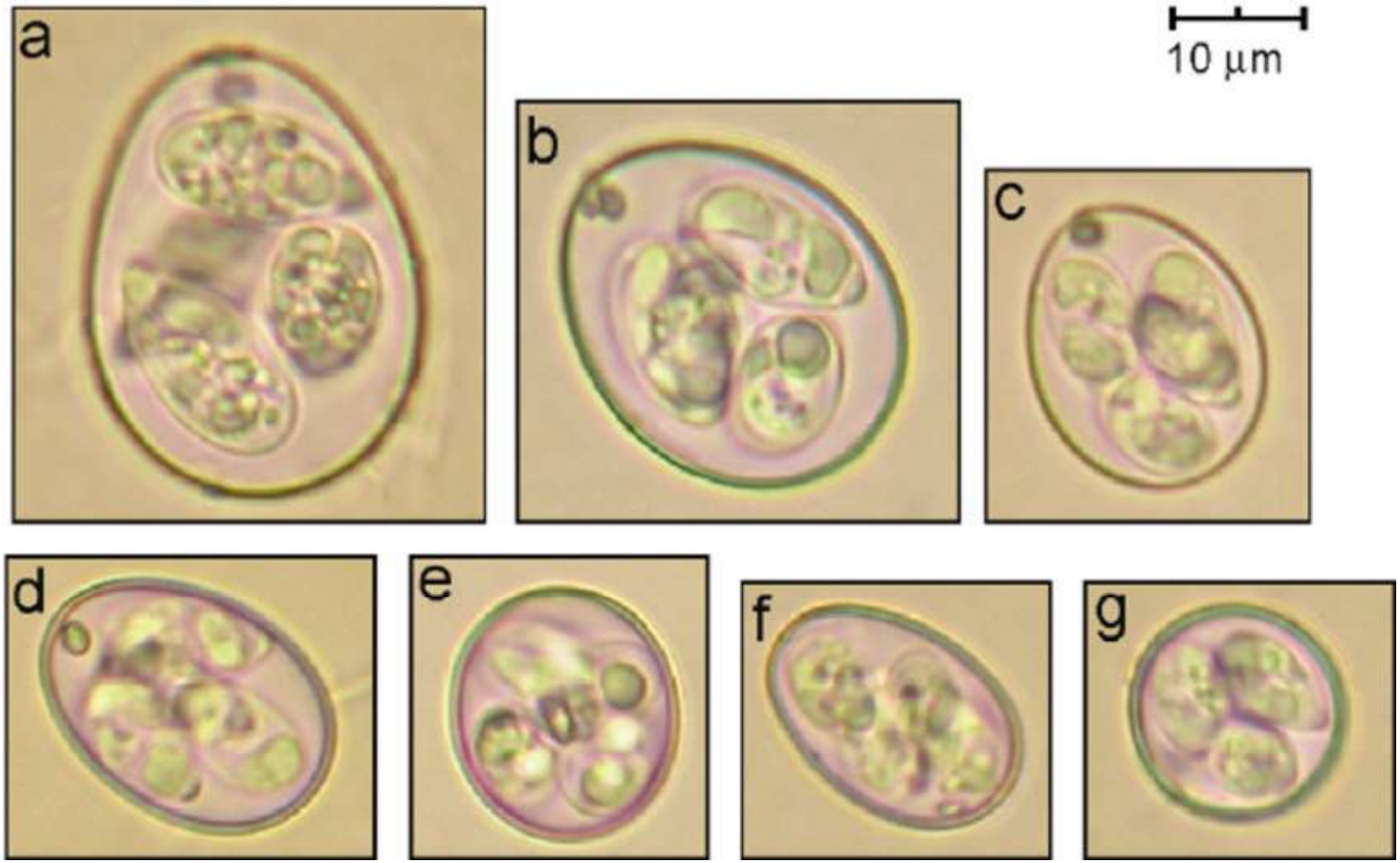


Fig. 1. Photomicrographs of oocysts of the seven Eimeria species of domestic fowl. Samples: (a) E. maxima , (b) E. brunetti , (c) E. tenella , (d) E. necatrix , (e) E. praecox , (f) E. acervulina , and (g) E. mitis .