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RESEARCH COMMUNICATION

OVERBERG RESEARCH PROJECT. IX. FIRST STAGE LARVAE PER GRAM [L_1p.g.] OF FAECES; AN EFFICIENT METHOD OF DIAGNOSING NEMATODE PARASITES OF SHEEP ANTE MORTEM

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ABSTRACT

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Nematode eggs were collected by mixing the faeces with a sugar solution, filling a flat-sided medicine bottle (100 mℓ), and allowing eggs to float and adhere to the upper surface for 30 min. After discarding the faeces, eggs hatched within 24 h at 20–30 °C. Each genus was counted in a counting chamber and the L_1p.g. estimated. The morphology of L_1 of *Haemonchus, Teladorsagia, Trichostrongylus, Oesophagostomum* and *Strongyloides* is briefly described.

INTRODUCTION

Whitlock (1959) described a method for harvesting first stage larvae (L_1 within 24 h of faeces being collected. In this paper Whitlock's method has been simplified. The number of L_1 per gram of each genus is estimated and the unhatched, embryonated nematode and *Nematodirus* eggs per gram counted separately.

MATERIALS AND METHODS

Harvesting first stage larvae

Flat-sided medicine bottle method (Whitlock, 1956).

Equipment

Wide-mouthed jar (100 mℓ Consul) with screw-cap and attached masking tape lable.

Polystyrene picnic hamper (Hebcooler) to keep faecal specimens at a stable temperature.

Ice-cubes in large plastic bag (380 × 280 mm).

Triple-beam balance (Ohaus) to determine the mass accurately in grams.

Teaspoon.

Tea sieve.

Large-mouthed glass jar (1 ℓ Consol).

Plastic, flat-sided medicine bottle (100 mℓ) with screw-cap.

Masking-tape specimen labels.

Sugar solution (2/3 sugar, 1/3 water).

Thermometer 0–100 °C.

Iodine solution (45 %.). Add hot water to 90 g KI and slowly dissolve 45 g of iodine crystals in this solution. Finally add water to obtain 1 ℓ of this solution.

Formalin 10 %, i.e. 4 % formaldahyde solution.

Counting chamber [Fig. 1 (M. Bohm, Hermanus)]. The lower Perspex (ICI) slide is 75 mm long × 35 mm broad with grids scored on the upper surface of each chamber, the outer dimensions of which are 12,5 × 4 mm. A line is scored down the centre, leaving 2 tracks in each slide 12,5 mm long × 2 mm wide. The 5 supporting bars are 2 mm thick and fixed to the slide approximately 12 mm apart. The cover slide is either a conventional glass slide (75 × 25 mm) or a Perspex slide of the same dimensions, fixed to the supporting bars and leaving an 8–10 mm ledge on one side of the lower slide. Fluid introduced into each chamber and L_1 and eggs lying on the grids represent the number present in 0,1 mℓ of the suspension.

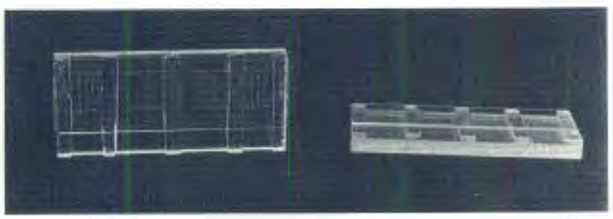

FIG. 1 Larval counting chambers for estimating L_1p.g. (M. Bohm, Hermanus) made of Perspex (ICI) top and side views. The outer dimensions of the rectangles scored on the upper surface of the lower slide are 12,5 × 4 mm with a line down the centre, dividing the rectangle into 2 equal rectangles 12,5 × 2 mm. The supporting bars between the base and cover-slide are 2 mm thick.

Compound microscope.

Incubator adjusted to 27–30 °C.

Harvesting of larvae

Collect faeces in the labelled jars. In hot weather, pack ice cubes around the jars in a plastic bag placed in the polystyrene container for transport to the laboratory. Mass-measure faeces to a maximum of 5 g, discard the excess, place the mass-measured faeces back in the labelled specimen jar and add 20 mℓ tap water. Leave for 30 min and break the softened pellets with a teaspoon, mixing the faeces with the water.

Place the tea sieve on top of the wide-mouthed jar (1 ℓ), pour the faecal mixture on to the sieve and wash it with a strong jet of cold water, until the fluid coming through the sieve is clear. Discard the residue on the tea sieve. Allow the faecal suspension to settle in the 1 ℓ jar for 5 min, discard the supernatant, leaving approximately 50 mℓ of sediment at the bottom of the jar.

Transfer the masking tape label to the flat side of the medicine bottle. Pour the sediment in the large jar into a small plastic funnel in the medicine bottle. If the sediment in the medicine bottle exceeds half the volume of the bottle after transfer, allow it to settle for another 5 min and discard the supernatant. Add sugar solution until the fluid overflows at the mouth of the bottle, leaving a convex meniscus. Screw the lid back on to the medicine bottle, avoiding air bubbles.

Lay the medicine bottle on a flat surface with the

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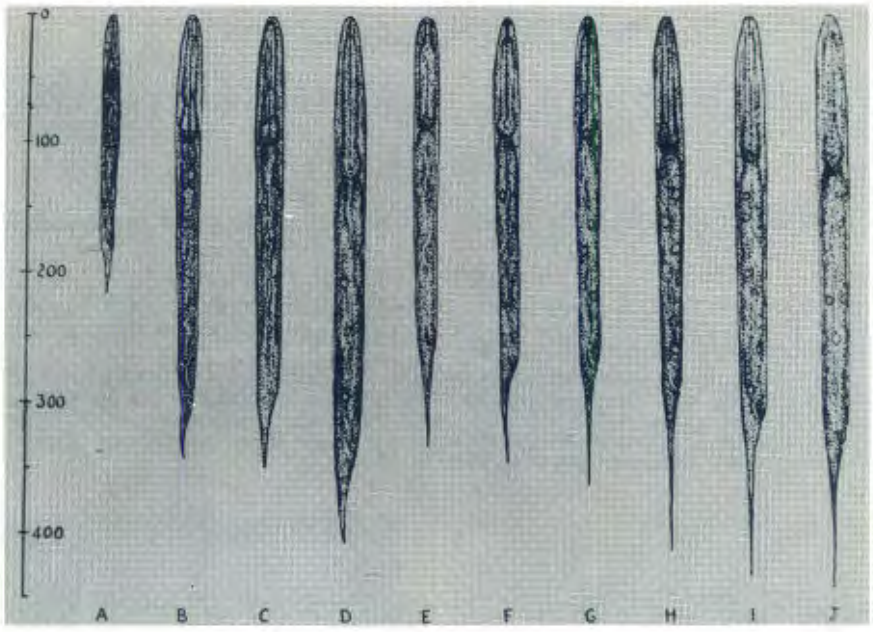

FIG. 2 First stage larvae. (A) *Strongyloides papillosus;* (B) *Trichostrongylus axei;* (C) *T. colubriformis;* (D) *Teladorsagia circumcincta* and *T. trifurcata;* (E) *Cooperia punctata* and *C. pectinata;* (F) *Haemonchus contortus;* (G) *H. placei;* (H) *Oesophagostomum columbianum;* (I) *Chabertia ovina;* (J) *Oesophagostomum venulosum* (from Whitlock, 1959).

label uppermost and leave for 30 min, allowing the eggs to float to and adhere to the inner, upper surface of the bottle. Pick up the medicine bottle, remove the lid and slowly (to avoid bubbles), pour out the faecal suspension into a sink or bucket, keeping the label uppermost.

Wash out the sludge at the bottom of the medicine bottle with a plastic wash bottle and avoid splashing.

Add 20 mℓ of water, shake vigorously 2 or 3 times after replacing the lid and place the medicine bottle on a flat surface with the label underneath. Eggs need oxygen to hatch and the water level in the medicine bottle should not exceed 5 mm in depth during incubation.

From spring to early autumn, as long as the minimum temperature exceeds 18 °C, the medicine bottle can be left on a shelf in the laboratory and the larvae will hatch within 24 h.

In the autumn and winter, incubate at 27–30 °C for 24 h. If no incubator is available, pour warm water at 40 °C into the polystyrene hamper (Hebcooler). Place the medicine bottle, with the label underneath, in the warm water and close the hamper. The next morning the temperature will probably have fallen to 20 °C, in which case the medicine bottle should be removed and the warm water replaced before returning the medicine bottle to the Hebcooler.

After 24 h incubation, remove the medicine bottle, shake, examine a drop microscopically to see whether the L_1 are present. If the eggs have not yet hatched, incubate for another 3–4 h until L_1 are present.

Transfer the label to a polytop bottle (25 mℓ) and pour the L_1 from the medicine bottle into the 25 mℓ bottle adding 2 drops of iodine solution and 2–3 drops of 10 % formaline to kill and fix L_1.

Adjust the volume in the polytop bottle to 1 g per 2 mℓ, e.g. 5 g faeces in 10 mℓ water.

Mix the larvae and eggs thoroughly by sucking the fluid into the Pasteur pipette and then squeezing the teat 2 or 3 times and filling the 1st chamber. Repeat the mixing process and fill the 2nd chamber. Count and identify each L_1 of *Haemonchus, Teladorsagia, Trichostrongylus, Oesophagostomum* and *Strongyloides*. Unhatched, embryonated nematode as well as *Nematodirus* eggs must be counted separately. Multiply the total L_1 of each species, unhatched, embryonated and *Nematodirus* eggs by 10 to obtain L_1 and eggs p.g.

If the 1st field is overcrowded with L_1, withdraw the fluid from both chambers, return to the polytop bottle and adjust the volume to 10 mℓ pipette, blow air vigorously in the water to mix the larval suspension, withdraw 1 mℓ and dilute to 10 or 20 mℓ with water. Fill and recount 2 chambers (2 × 0,1 mℓ) and estimate the total number of L_1 in 1 mℓ (i.e. × 50 or × 100), multiplying this total by 2 to give the L_1p.g. of faeces.

Identification of 1st stage larvae (Fig. 2)

Measurements of the larvae are listed in Table 1 (Whitlock, 1959, cited by Reinecke 1983) and the genera are illustrated in Fig. 2 (Whitlock, 1959).

The morphology resembles that of the 3rd stage larvae (L_3) of the same genus. These are differentiated from each other on total length and the length of the tail.

Short larvae

Strongyloides: These ae the shortest larvae and do not exceed 216 μm. The oesophagus is proportionately longer than that of the other larvae and the tail is very short.

Trichostrongylus: The mean length of these larvae is between 340 and 351 μm, with a short tail and a slender body.

Haemonchus: This has the same length as *Trichostrongylus* (345 μm) but has a medium length, fine whip-like tail which is 75 μm long.

Long larvae

Teladorsagia: Mean length more than 400 μm.

TABLE 1 Dimensions of first-stage nematode larvae (from Whitlock, 1959)

	Total length (μm)	Width (μm)		Length of anus to tip of tail (μm)	
	Range	Mean	Mean	Range	Mean
Chabertia ovina	381,3 – 479,7	430,3	23,4	101,5 – 129,1	116,2
Cooperia pectinata and *C. punctata*	301,4 – 356,7	330,3	17,1	68,2 – 87,0	76,6
Haemonchus contortus	307,5 – 387,5	344,6	18,8	69,6 – 84,1	74,9
H. placei	307,5 – 418,2	362,8	19,3	81,2 – 101,5	92,3
Oesophagostomum columbianum	356,7 – 467,4	412,7	20,5	107,3 – 132,0	120,1
O. venulosum	399,8 – 492,0	441,1	25,2	100,1 – 116,0	107,5
Teladorsagia circumcincta and *T. trifurcata*	362,9 – 461,3	404,8	20,4	52,2 – 68,2	61,0
O. ostertagi	300,0 – 500,0	—	—	—	—
Strongyloides papillosus	172,2 – 252,5	216,4	—	—	—
Trichostrongylus axei	289,1 – 387,5	342,2	19,2	52,2 – 63,8	57,4
T. colubriformis	304,5 – 405,9	351,4	18,1	50,8 – 66,7	58,6

These larvae have a short tail but the body is longer and wider than that of the other genera mentioned above.

Oesophagostomum: These are the longest larvae, exceeding 410 μm in length, with a very long whip-like tail. (90–120 μm). The body is broad but the worm is very rarely straight, being either curved or twisted, and only with experience can the total length be estimated.

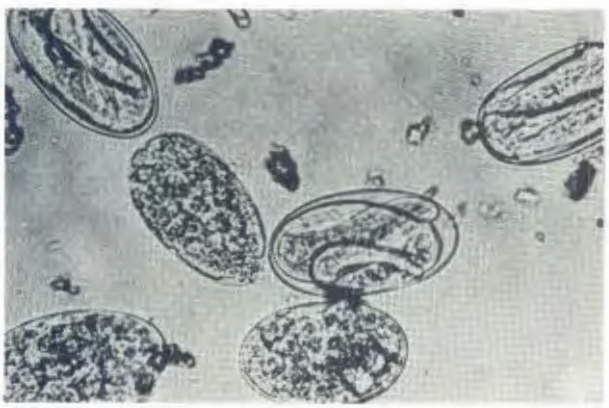

FIG. 3 Unhatched and embryonated eggs (from Whitlock, Kelley, Porter, Griffen & Martin 1980).

DISCUSSION

Sterile eggs (Fig. 3)

The first thing I noticed when using this technique was the frequent presence of morulated and embryonated eggs. These were particularly noticeable during a trial, when faeces were collected from lambs grazing on the same pastures as ewes. The latter had been dosed with a sustained release device containing a benzimidazole. Despite the fact that the lambs had received no anthelmintic, the percentage of eggs exceeded 60 in some specimens, when compared with total L_1 and eggs harvested.

Since the main object of this technique was to arrive at a generic diagnosis, I suggest that any unhatched eggs be regarded as "sterile eggs".

A notable exception is the egg of *Nematodirus* which is readily diagnosed and only hatches under optimal conditions after 7–10 days. Therefore these eggs should be recorded separately from the others.

Diagnosis within 24 h

The biggest advantage of this technique is the ability to diagnose the genera present and the number of each genus per gram in as little as 24 h. Another advantage of the L_1p.g. method is that a small mass of faeces can be used to make a diagnosis, compared with the standard worm egg count in which 2 g are required for an egg count and another 5 g or more for a culture of L_3. Furthermore, counting eggs, harvesting 3rd stage larvae (L_3) 7–10 days later and then converting the original egg count to the various genera present is a laborious time-consuming process. It is also based on a false premise, i.e. that all the eggs in the original egg count are fertile and will hatch and develop through to the L_3. The number of L_3 harvested may not have any relationship to the original egg count.

Conversion to L_1p.g. with less than 5 g of faeces

It is rarely possible to collect 5 g of faeces from suckling lambs and only 1 or 2 pellets or a small quantity of liquid faeces may be present in the rectum of adult animals. In anthelmintic trials (e.g. faecal L_1p.g. reduction tests) it is essential to collect faeces from each individual sheep, earmarked either as undosed controls or those allocated to treated groups, and some of these sheep have no faeces in the rectum. An enema may only yield a few pellets. If the total L_1 counted in these small faecal quantities

TABEL 2 Arithmetic conversion: total L_1 to L_1p.g. in faecal specimens mass-measuring less or more than 1 g

Faecal mass g	Conversion to L_1p.g.	Faecal mass g	Conversion to L_1p.g.
0,1	× 10,0	2,7	× 0,37
0,2	× 5,0	2,8	× 0,36
0,3	× 3,3	2,9	× 0,34
0,4	× 2,5	3,0	× 0,33
0,5	× 2,0	3,1	× 0,32
0,6	× 1,7	3,2	× 0,31
0,8	× 1,25	3,3	× 0,30
0,9	× 1,11	3,4	× 0,29
1,0	× ≡	3,5	× 0,29
1,1	× 0,91	3,6	× 0,28
1,2	× 0,83	3,7	× 0,27
1,3	× 0,77	3,8	× 0,26
1,4	× 0,71	3,9	× 0,26
1,5	× 0,67	4,0	× 0,25
1,6	× 0,63	4,1	× 0,24
1,7	× 0,59	4,2	× 0,24
1,8	× 0,56	4,3	× 0,23
1,9	× 0,53	4,4	× 0,23
2,0	× 0,50	4,5	× 0,22
2,1	× 0,48	4,6	× 0,22
2,2	× 0,45	4,7	× 0,21
2,3	× 0,43	4,8	× 0,21
2,4	× 0,42	4,9	× 0,20
2,5	× 0,40	5,0	× 0,20
2,6	× 0,38		

is multiplied by the appropriate figure given in Table 2, the L_1p.g. can be estimated.

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