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RECOVERY OF INFECTIVE 3RD STAGE LARVAE OF HAEMONCHUS CONTORTUS AND OSTERTAGIA OSTERTAGI BY MIGRATION IN AGAR GEL

By

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MWEGOHA, WILLIAM M. and ROLF JESS JØRGENSEN: *Recovery of infective 3rd stage larvae of Haemonchus contortus and Ostertagia ostertagi by migration in agar gel.* Acta vet. scand. 1977, *18*, 293—299. — A laboratory technique is described for the recovery of Haemonchus contortus and Ostertagia ostertagi infective larvae by migration in agar gel. The addition of bile increased the recovery rate of the haemonchus larvae, but had a somewhat depressive effect on the number of ostertagia larvae recovered. Similarly, storage at 4°C lowered the yield of larvae of both species, compared to freshly harvested larvae. However, the recovery rates for both species were sufficiently high to recommend the technique for isolation of the infective stages from field samples.

Haemonchus contortus larvae; Ostertagia ostertagi larvae; recovery; agar gel; bile.

Quantitation of infective larvae from herbage has become an essential part of basic studies on the epidemiology of dictyocaulus and trichostrongylid infections. It provides important information about the contamination, distribution and seasonal occurrence of infective larvae in the field. This knowledge is needed for the development of better control measures against parasitic infections in grazing ruminants and for evaluation of new management systems.

One problem in the technique has been the lack of an efficient method for the extraction of the larvae from the pasture samples.

Several methods have been reported (*Taylor* 1939, *Parfitt* 1955, *Durie* 1959, *Donald* 1967, *Lancaster* 1970). These techniques have been based mainly on two principles, firstly the specific gravity of the larvae (by flotation, sedimentation and centrifugation) and, secondly, the particle size of the larvae (by sieving). The recovery rates have varied and the main disadvantage, as mentioned by *Jørgensen* (1975 b), is the fact that there is an overlapping in both the specific gravity and particle size of the parasite larvae and those of soil nematodes and debris. Other methods have depended on the activity of the larvae themselves for isolation (*Taylor, Persson* 1974). Attempts have also been made to modify the apparatus (*Durie, Persson*), but still the results were variable or the methods less suitable for routine purposes.

The following experiments were designed to observe the migration of trichostrongylid infective larvae in agar gel, employing the technique described by *Jørgensen* (1975 b) for the isolation of dictyocaulus larvae. The purpose was to study whether trichostrongylid larvae can migrate in agar gel similarly to dictyocaulus larvae (*Jørgensen* 1975 a) and whether the larvae can be recovered in sufficiently high percentage so that the method can be recommended for the examination of pasture samples for these parasitic larvae.

The experiments were carried out with and without the addition of ox bile to the agar gel. Bile was used for comparison of its effect on the rate of recovery, since bile has been shown to have a stimulatory effect on dictyocaulus larvae (*Jørgensen* 1973).

The experiments comprised pure laboratory cultures of Haemonchus contortus and Ostertagia ostertagi.

MATERIALS AND METHODS

Haemonchus contortus

Adult female haemonchus worms were isolated from sheep abomasa obtained at a slaughter-house in southern Jutland (Tønder), December 1974. The worms were crushed to release the eggs which were incubated at 26°C for eight days in sterile faeces mixed with sawdust. The third stage larvae were extracted by a modified Baermann technique (*Henriksen* 1965) and were tested either immediately or after storing in shallow water at 4°C.

Ostertagia ostertagi

Third stage larvae of these organisms were obtained by culturing faeces, as described above, from calves which were infected with a monoculture obtained at the State Veterinary Serum Laboratory.

The procedure and the material and apparatus described by *Jørgensen* (1975 b) for the isolation of dictyocaulus larvae were used in the present recovery experiments.

Glass columns

Glass columns with a total length of 385 mm were used. They consisted of a cylindrical part 305 mm long and 43 mm in diameter tapering to a pipe stub with an inner diameter of 2 mm. The pipe stub was fitted with rubber tube and clamp. The tubes were kept vertically in racks.

Trays

Plastic trays with a smooth surface and plain bottom measuring 200 × 300 mm were used.

Experimental procedures

Agar gel, 3 %, was prepared from Difco Bacto Agar and kept in a water bath at 50°C. Ox bile was also prepared and kept in the same water bath.

Measured equal amounts of larval suspension were placed in six small counting dishes. The number of viable larvae in each dish was counted and the contents were transferred to 250 ml beakers to which 40°C warm water was added to the level of 60 ml.

When freshly harvested larvae were tested, the method of counting live active larvae before processing was found to be difficult because of their fast movements and the procedure was therefore modified. In each dish of two parallel rows of 12 counting dishes 2 ml of a uniform suspension of larvae were placed. Twelve of the dishes were processed for larval migration as described above (six with bile and six without bile), without counting. The other 12 were used for counting after killing the larvae with Lugols iodine. The mean number of larvae per dish was used in the calculations.

A set of six glass columns was prepared with water at 40°C. A 20 cm by 35 cm piece of cloth gauze was fixed in each of six plastic trays, corresponding to the number of samples for processing, by a fine spray of water.

Sixty ml of the liquid agar was mixed with 15 ml of prewarmed bile and poured into each of three beakers. Similarly, 60 ml of agar plus 15 ml of water was poured into three beakers. All beakers with their contents were kept at 40°C until further processing. Each warm liquid agar mixture was then quickly mixed with a larval suspension and poured into a tray.

The agar mixture was left for 15 min., then the solid slab, approx. 2.5 mm thick, was lifted from the tray, held hanging vertically, and wound into a roll which was then submerged in water in a glass column. Incubation took place at 38°C overnight.

Approx. 10 ml of fluid was drawn off into conical centrifuge tubes from each column and centrifuged at 2,000 r.p.m. for 2 min. The supernatant was syphoned off and the larval sediment transferred to counting dishes. Drops of Lugols iodine were added to each dish; they were left for about 1 hr. and then discoloured by sodium thiosulphate. The third stage infective larvae retained the red iodine stain because of their thick cuticle through which diffusion apparently takes place at a very low rate. The recovered larvae were counted under the stereomicroscope.

RESULTS

The results are presented in Tables 1, 2 and 3. It appears that the infective larvae of both species were capable of migration in agar gel since a large proportion could be recovered.

Table 1 shows that an average of 53.5 % (range 46—59 %) of old haemonchus larvae were recovered when processed without bile. The addition of bile consistently increased the recovery rate resulting in a mean recovery of 81.5 % (range 67—90 %).

Table 2 shows that freshly harvested third stage haemonchus larvae could be recovered in comparably high numbers even without the addition of bile (72 %), probably due to a higher level of spontaneous motility in these larvae. When larvae were stored for six days, the recovery rate was found to be comparable to that of the old larvae.

Table 2 also shows that as many as 90 % of freshly harvested infective larvae of Ostertagia ostertagi were recovered without

the addition of bile, and that the addition of bile lowered the recovery rate by 20 %. Storage of larvae for six days as well as for more than six months (Table 3) lowered the recovery rates. The effect of bile remained slightly depressive.

Table 1. Recovery of old Haemonchus contortus larvae. Larvae stored at 4°C for more than 6 months.

Trial	Agar gel with bile			Agar gel without bile		
	number added	number recovered	% recovered	number added	number recovered	% recovered
1	44	26	59	44	18	41
	34	22	65	35	16	46
	52	40	77	35	18	51
	Mean recovery		67 %	Mean recovery		46 %
2	152	140	92	112	65	58
	229	189	83	162	72	44
	185	176	95	142	72	51
	Mean recovery		90 %	Mean recovery		51 %
3	95	75	79	100	59	59
	106	90	85	109	67	61
	80	70	88	93	53	57
	Mean recovery		84 %	Mean recovery		59 %
4	102	78	77	115	57	50
	96	82	85	116	83	72
	110	102	93	90	48	53
	Mean recovery		85 %	Mean recovery		58 %

Table 2. Recovery of young 3rd stage larvae. Mean figures ± s of six determinations.

Age (number of days stored at 4°C)	Initial number of larvae	Larvae recovered in agar gel			
		with bile		without bile	
		number recovered	mean recovery %	number recovered	mean recovery %
Haemonchus contortus					
0	82 ± 9	70 ± 10	85	60 ± 13	72
6	103 ± 6	72 ± 5	70	44 ± 5	43
Ostertagia ostertagi					
0	64 ± 10	46 ± 7	70	59 ± 13	90
6	116 ± 7	75 ± 7	65	87 ± 11	75

Table 3. Recovery of old Ostertagia ostertagi larvae. Larvae stored at 4°C for more than 6 months. Mean figures ± s of 12 determinations.

Trial	Initial number of larvae	Larvae recovered in agar gel			
		with bile		without bile	
		number recovered	mean recovery %	number recovered	mean recovery %
1	69 ± 11	32 ± 5	47	36 ± 7	52
2	66 ± 6	28 ± 7	43	36 ± 6	55
3	78 ± 12	31 ± 8	41	55 ± 11	71

DISCUSSION

It is interesting to note that the addition of bile increased the recovery rate of the Haemonchus contortus larvae, but somewhat lowered the recovery rate of the Ostertagia ostertagi larvae. The reason for this difference in response between the infective stages of these two abomasal parasites is unknown.

Despite the difference in response to bile and despite the differences in recovery rates, a sufficiently large proportion of the larvae are recovered to recommend the described technique for field use. *Jørgensen* (1975 b) pointed out the advantages of isolating infective dictyocaulus larvae by migration in agar gel. With the present results it is concluded that the technique, with the addition of bile, is suitable for isolation of the infective stages of Ostertagia ostertagi, Haemonchus contortus, Dictyocaulus viviparus and Dictyocaulus filaria.

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SAMMENDRAG

Isolering af infektive tredie stadium larver af Haemonchus contortus og Ostertagia ostertagi ved hjælp af migration i agar gel.

En teknik baseret på migration i agar gel indeholdende galde blev afprøvet med henblik på isolering af de infektive stadier af trichostrongyliderne Haemonchus contortus og Ostertagia ostertagi. Effekten af galdetilsætning og effekten af de anvendte larvers alder belyses. Galdetilsætning øgede genfindelsesprocenten af haemonchus larverne, hvorimod effekten på ostertagia larverne var modsat, men mindre udtalt. Ligeledes fandtes lavere genfindelsesprocenter for larver af begge arter efter opbevaring ved 4°C. Det konkluderes, at migration i agar gel vil kunne anbefales til isolering af infektive haemonchus og ostertagia larver fra feltprøver.

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