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# RECOVERY OF NEMATODES FROM RUMINANTS BY MIGRATION FROM GASTRO-INTESTINAL INGESTA AND MUCOSA GELLED IN AGAR: PRELIMINARY REPORT

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ABSTRACT

VAN WYK, J. A. & GERBER, H. M. 1978. Recovery of nematodes from ruminants by migration from gastro-intestinal ingesta and mucosa gelled in agar: preliminary report. *Onderstepoort Journal of Veterinary Research*, 45, 29–38 (1978).

When gastro-intestinal ingesta and mucosa containing larvae or adult *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus colubriformis, Strongyloides papillosus, Nematodirus spathiger, Gaigeria pachyscelis, Oesophagostomum columbianum* or *Chabertia ovina* were suspended in 0,75–1,00% agar gel and incubated in physiological saline, a mean of 93,6% of the helminths migrated from the agar-ingesta or agar-mucosa into the physiological saline.

The lowest suitable concentration of agar was 0,85–0,90% for one batch of agar and 0,65% for another.

Although most of the worms had migrated from the agar gel after 3–4 h of incubation, migration usually continued for longer than 7 h.

While low concentrations of formalin partially inhibited migration from the gel, slightly more worms were recovered from agar containing 1% bile than from agar alone.

*Résumé*

*RAPPORT PRÉLIMINAIRE CONCERNANT LA RÉCUPÉRATION DE NÉMATODES DE RUMINANTS PAR MIGRATION À PARTIR DE MUQUEUSES ET INGESTA GASTRO-INTESTINAUX PRIS DANS UN GEL D'AGAR*

*Lorsque des muqueuses et des ingesta gastro-intestinaux contenant des larves ou des adultes de* Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus colubriformis, Strongyloides papillosus, Nematodirus spathiger, Gaigeria pachyscelis, Oesophagostomum columbianum *ou* Chabertia ovina *ont été mis en suspension dans un gel d'agar à 0,75–1% et incubés dans une solution physiologique saline, 93,6% des helminthes, en moyenne, ont migré de l'agar-ingesta ou de l'agar-muqueuse vers la solution physiologique.*

*On a constaté que la plus faible concentration adéquate d'agar était de 0,85–0,9% pour un lot d'agar et 0,65% pour un autre.*

*Bien que la plupart des vers eussent quitté le gel d'agar après 3 ou 4 heures d'incubation, la migration s'est généralement prolongée pendant plus de 7 heures.*

*Tandis que de faibles concentrations de formol ont partiellement inhibé le mouvement migrateur à partir du gel, on a récupéré un peu plus de vers à partir d'agar contenant 1% de bile qu'à partir d'agar seul.*

## INTRODUCTION

In this laboratory total worm recoveries are routinely carried out on experimental ruminants. The adults of most species may be recovered macroscopically, but the larval stages and some adults can only be detected microscopically. The examination of large volumes of ingesta and the manual removal of individual worms are both tedious and costly. A simplified, alternative method of recovery has become an urgent necessity.

Jørgensen (1975) embedded herbage washings containing *Dictyocaulus* spp. infective larvae (L3) in 1,3% agar gel and by this method recovered the larvae which had migrated during prolonged incubation. A modification of this technique was used to recover parasitic larval and adult nematodes from gastro-intestinal ingesta and mucosa of sheep.

## GENERAL MATERIALS AND METHODS

### *Larvae and hosts*

The infective larvae (L3) used in these investigations originated from pure strains of nematodes maintained in the laboratory as described by Reinecke (1973).

Dorper sheep, maintained worm-free since birth, were used in all the experiments. Some sheep were infested more than once with the same species. Since, in every case, slaughter was planned so that adult worms originated only from the first infestation, the age of the adult worms was accurately known, but some larvae may have originated from either the primary or subsequent infestations. The various nematode species at all developmental stages were recovered simultaneously from each sheep from the same preparation. However, in some instances the different developmental stages were considered separately and some sheep were included in more than one experiment.

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### *Aliquots of ingesta and mucosa*

The gastro-intestinal contents, obtained as described by Reinecke (1973), were divided into convenient but unequal aliquots. Each aliquot was regarded as a separate entity, suitable only for comparing the numbers of worms that migrated from the agar gel with those that failed to do so.

In Experiment VII, however, the stripped and blended mucosa was thoroughly mixed and divided into aliquots of equal mass for the comparison of the number of worms recovered.

### *Preparation of the agar slabs and recovery of the worms*

Jørgensen (1975) made agar slabs by pouring warm agar/worm suspension in a thin film onto a cloth laid on a smooth-bottomed plastic tray and allowing it to cool to a gel. By allowing the cloth to protrude from one side of the gel, it was possible to roll the slab into a cylinder and suspend it in a water-filled glass cylinder for larval recovery. We found that only very small volumes of suspension could be processed by this technique since thick agar sheets cracked and disintegrated when rolled up, and we therefore introduced the following modifications.

Plastic insect mesh with approximately 1,2 mm apertures was framed by perspex strips to form a rectangle 37 cm × 24 cm × 1,2 cm. The perspex strips forming the upper part of the frame were 1 cm thick,

and those of the lower part 0,3 cm thick. Another piece of perspex, 34,5 cm × 21,5 cm × 0,3 cm, formed the base-plate which fitted snugly into the lower part of the frame. When the base-plate was in position it formed a shallow, mesh-lined mould (Fig. 1).

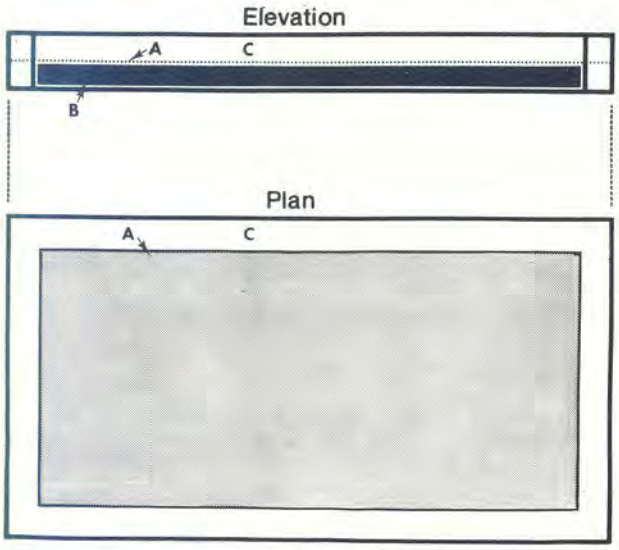


FIG. 1 The plan of the frame used for preparing slabs of agar. A=plastic mesh; B=base-plate; C=perspex strips forming the frame

Agar solution maintained at 45–50 °C was cooled to 38–40 °C immediately prior to being mixed with aliquots of ingesta. After further cooling to 34 °C, the mixture was poured into the mould formed by the frame and base-plate. The frame with the agar slab supported on the sieve was then lifted off the base-plate and submerged in physiological saline for incubation at 40 °C. In Experiments III, IV and VII, the joints between the base-plate and frame were sealed with a small amount of agar before the mixture was poured into the mould.

Agar solutions for Experiment I were prepared with tap water but physiological saline (made up with tap water) was used for Experiments II–VIII.

Two methods were used to prepare agar slabs with a final estimated concentration of 0,75–1%. For Sheep 1, 2, 3, 7, 10 and 11, excess moisture was removed by manual pressure on a 37 μm aperture sieve, and it was assumed that no dilution occurred when the aliquot was added to the agar.

For Sheep 4–6, 8,9 and 12–15, unsieved aliquots of ingesta were mixed with an equal volume of agar solution at double the required concentration. In this instance it was assumed that the final concentration of agar was 50% of the initial strength, although the ingesta probably influenced the concentration to some extent.

In all the investigations but the first (to determine the rate of migration from agar), worms lying loose on slabs after incubation were gently rinsed off after 6 h, the slabs transferred to fresh saline solution and rinsed again after 24 h. In the first investigation (to determine the rate of migration of worms from agar), rinsing was repeated hourly for 7 hours and again after 24 h. Worms recovered at each incubation interval were counted separately.

After 24 hours of incubation, the agar slab was broken manually and washed into a bucket with a strong jet of water. The crumbled gel was melted and immediately washed on a 37 μm aperture sieve to remove the agar and fine faecal particles. The residue on the sieve was examined microscopically for worms that had failed to migrate from the agar during incubation.

The number of worms of each species and their stage of development were determined by identifiying the first 50 worms recovered from each suspension. If fewer than 50 worms were present, they were all identified.

### *I. The rate of migration of* Haemonchus contortus *and* Oesophagostomum columbianum *from gelled agar*

*Method*

Sheep 1 was infested on 2 occasions with both *H. contortus* and *O. columbianum,* 35 days and 15 days before slaughter.

Two aliquots of abomasal ingesta and one of large intestinal ingesta were mixed separately with agar to give a final concentration of 0,75% and 0,80% for abomasal and 0,80% for large intestinal ingesta.

The numbers of worms recovered at hourly intervals for 7h and again after 24 h are summarized in Table 1.

*Results* (Table 1)

After 24 hours of incubation, 3,7% adult and 4,6% immature *H. contortus* and 4,3% adult and 4,8% immature *O. columbianum* had not migrated from the agar.

After 2 h of incubation, 93% adult *H. contortus* had migrated from the agar, but it took another 2 h for a similar percentage of larvae to migrate. More than 90% of adult *O. columbianum* had migrated after 2 h while the few larvae were all recovered after 1 h.

Worms that migrated from 0,80% agar were almost free from ingesta, but some contamination occurred with 0,75% agar, which was friable and crumbled during handling. Friability of the agar increased in proportion to the amount of ingesta added.

*Comment*

Larvae of *H. contortus* migrated more slowly than adults, but almost all were recovered after 7 h of incubation.

In every instance a higher but statistically not significant percentage of adult worms was recovered than larvae.

In 2 out of 6 aliquots, migration continued after 7 h, and it was decided to incubate for 24 hours in further trials.

The 0,75% agar gave comparatively poorer results because of its friability, a condition probably aggravated by the repeated rinsing. As mentioned previously, the agar may have been diluted by addition of the ingesta and it may be necessary to standardize the amount of solid material added to the agar to correct this tendency.

### *II. Migration of* H. contortus *and* O. columbianum *from various concentrations of agar*

*Method*

Sheep 2 and 3 were infested with *H. contortus* and *O. columbianum,* 39 and 41 days respectively before slaughter. Aliquots of ingesta containing the worms were gelled in different concentrations of agar, as shown in Table 2, and incubated for 24 h.

TABLE 1 The rate of migration of *H. contortus* and *O. columbianum* from agar

| Incubation (hours) | Number of worms recovered | | | | | |
|---|---|---|---|---|---|---|
| | Adults | | | Immatures* | | |
| | *H. contortus* | | *O. columbianum* (0,80% agar) | *H. contortus* | | *O. columbianum* (0,80% agar) |
| | 0,80% agar | 0,75% agar | | 0,80% agar | 0,75% agar | |
| 1 | 214 | 206 | 99 | 215 | 194 | 20 |
| 2 | 22 | 14 | 30 | 63 | 49 | 0 |
| 3 | 7 | 5 | 3 | 37 | 34 | 0 |
| 4 | 2 | 10 | 3 | 17 | 25 | 0 |
| 5 | 4 | 0 | 0 | 10 | 14 | 0 |
| 6 | 2 | 2 | 0 | 6 | 2 | 0 |
| 7 | 0 | 1 | 0 | 1 | 1 | 0 |
| 24 | 0 | 1 | 0 | 0 | 2 | 0 |
| Total** | 251 | 239 | 135 | 349 | 321 | 20 |
| In agar (%) | 3,8 | 3,6 | 4,3 | 4,6 | 4,5 | 4,8 |

* L4 *O. columbianum* and L4 and 5th stage *H. contortus*
** The total number of worms which migrated out of the agar

TABLE 2 Migration of *H. contortus* and *O. columbianum* from different concentrations of agar

| Sheep | Agar concentration (%) | Numbers of worms | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *H. contortus* | | | | *O. columbianum* | | | |
| | | Adults | | Larvae | | Adults | | Larvae | |
| | | Total No. | In agar (%) | Total No. | In agar (%) | Total No. | In agar (%) | Total No. | In agar (%) |
| 2 | 0,75 | 315 | 1,6 | 9 | 0,0 | 83 | 4,8 | — | — |
| 2 | 0,80 | 370 | 4,6 | 10 | 0,0 | 181 | 1,1 | 5 | 0,0 |
| 3 | 0,85 | 383 | 11,5 | — | — | 119 | 2,6 | — | — |
| 3 | 0,90 | —* | —* | — | — | 55 | 5,5 | — | — |
| 3 | 0,95 | — | — | — | — | 150 | 4,0 | — | — |
| 2 | 1,00 | 323 | 9,0 | 4 | 0,0 | — | — | — | — |

* This concentration was tested but, as the agar plate was dropped, the preparation was damaged

*Results*

The results are summarized in Table 2.

The percentage of adult *H. contortus* that did not migrate from the agar varied from 1,6%–11,5%, while from 1,1%–5,5% of the adult *O. columbianum* remained in the agar.

All the larvae which were present in small numbers in some of the aliquots of ingesta migrated from the gel.

*Comment*

The migration of adult *H. contortus* from high concentrations of agar was poorer than from the lower concentrations. However, in the light of similar variations in migration in the other investigations reported on in this paper this was probably not important.

Since the lowest concentration of agar which did not crumble when handled was 0,85%, either 0,85% or 0,90% agar was used in subsequent trials, except in Experiment IV in which a new batch of agar dit not crumble at a final concentration of 0,65%. The fact that the 0,75% agar was not as friable as in Experiment I may indicate that handling and dilution probably contribute to crumbling. As there may be an inverse relationship between the gel strength of the agar and the migration of the nematodes, lower concentrations of agar should be retested under strictly controlled conditions and the concentrations exactly determined.

Jørgensen (1975) reported that from 19,0%–41,6% of *Dictyocaulus* L3 failed to migrate from 1,3% agar gel. The relatively low recoveries were possibly due to the use of a higher concentration of agar than that tested in the present trials.

Larval recoveries were very encouraging, but no meaningful conclusions could be arrived at since the numbers were too small.

*III. Migration from different thicknesses of agar*

*Method*

Sheep 4 was infested on 3 occasions with L3 *H. contortus,* 23, 8 and 2 days before being killed.

Aliquots of abomasal ingesta were made up to various volumes with saline and thereafter each was mixed with an equal volume of 1,8% agar to give a final concentration of 0,9% agar. The thickness of the agar gel slab for each aliquot is listed in Table 3.

TABLE 3 Migration of *H. contortus* from agar slabs of various thicknesses

| Thickness of agar (mm) | Adult worms | | | 4th stage larvae | | |
|---|---|---|---|---|---|---|
| | Total No. | Failed to migrate | In agar (%) | Total No. | Failed to migrate | In agar (%) |
| 8 | 134 | 8 | 6,0 | 287 | 0 | 0,0 |
| 8 | 149 | 10 | 6,7 | 114 | 10 | 8,8 |
| 8 | 130 | 12 | 9,2 | 38 | 6 | 15,8 |
| **Mean** | **138** | **10** | **7,3** | **146** | **5** | **3,6** |
| 6 | 105 | 10 | 9,5 | 100 | 10 | 10,0 |
| 6 | 144 | 0 | 0,0 | 73 | 5 | 6,9 |
| 6 | 136 | 15 | 11,0 | 63 | 0 | 0,0 |
| **Mean** | **128** | **8** | **6,5** | **79** | **5** | **6,4** |
| 4 | 47 | 0 | 0,0 | 131 | 0 | 0,0 |
| 4 | 71 | 5 | 7,0 | 126 | 5 | 4,0 |
| 4 | 82 | 5 | 6,1 | 31 | 5 | 16,1 |
| **Mean** | **67** | **3** | **5,0** | **96** | **3** | **3,5** |
| 2 | 43 | 5 | 11,6 | 23 | 5 | 21,7 |
| 2 | 27 | 0 | 0,0 | 3 | 0 | 0,0 |
| 2 | 36 | 0 | 0,0 | 22 | 5 | 22,7 |
| **Mean** | **35** | **2** | **4,7** | **16** | **3** | **20,8** |
| **Mean** | — | — | **6,3%** | — | — | **5,0%** |

TABLE 4 Migration of *H. contortus* adults after various time intervals between autopsy and the processing of the ingesta

| Hours at room temp. | Temp. of aliquot | Total adults | Adult worms in agar (%) | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | Mean |
| 0,5 | 24,0 °C | 480 | 8,3 | 0,0 | 6,3 | 4,2 |
| 1,0 | 20,0 °C | 542 | 7,8 | 13,0 | 11,5 | 11,1 |
| 1,5 | 18,5 °C | 676 | 6,4 | 11,2 | 11,8 | 10,4 |
| 2,5 | 18,0 °C | 655 | 13,3 | 12,8 | 17,4 | 14,5 |
| 3,5 | 16,5 °C | 863 | 26,2 | 12,7 | 19,1 | 20,3 |
| 4,5 | 16,4 °C | 617 | 19,3 | 20,9 | 17,8 | 19,4 |

TABLE 5 Migration of L4 *H. contortus* after various time intervals between autopsy and the processing of the ingesta

| Hours at room temp. | Temp. of aliquot | Total L4 | Larvae in agar (%) | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | Mean |
| 0,5 | 24,0 °C | 91 | 0,0 | 0,0 | 0,0 | 0,0 |
| 1,0 | 20,0 °C | 35 | 16,1 | 0,0 | 0,0 | 15,3 |
| 1,5 | 18,5 °C | 76 | 13,5 | 0,0 | 0,0 | 6,6 |
| 2,5 | 18,0 °C | 91 | 0,0 | 0,0 | 0,0 | 0,0 |
| 3,5 | 16,5 °C | 22 | 0,0 | 0,0 | 0,0 | 0,0 |
| 4,5 | 16,4 °C | 37 | 0,0 | 0,0 | 0,0 | 0,0 |

TABLE 6 Migration of adult *T. colubriformis* after various time intervals between autopsy and the processing of the ingesta

| Hours at room temp. | Temp. of aliquot | Total worms | Adult *T. colubriformis* in agar (%) | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | Mean |
| 0,5 | 24,0 °C | 127 | 0,0 | 0,0 | 0,0 | 0,0 |
| 1,0 | 20,0 °C | 171 | 0,0 | 0,0 | 0,0 | 0,0 |
| 1,5 | 18,5 °C | 141 | 0,0 | 15,6 | 0,0 | 3,5 |
| 2,5 | 18,0 °C | 183 | 0,0 | 7,9 | 0,0 | 2,7 |
| 3,5 | 16,5 °C | 100 | 0,0 | 0,0 | 13,5 | 5,0 |
| 4,5 | 16,4 °C | 128 | 18,9 | 0,0 | 43,5 | 15,6 |

While total counts were made of the worms that migrated from the agar, with 2 exceptions only, one 20% aliquot of each sample of ingesta was examined for those that failed to migrate from the agar.

*Results*

The results are summarized in Table 3.

A mean of 7,3%/3,6% (adult/L4 *H. contortus*) failed to migrate from the 8 mm thick agar slabs and 6,5%/6,4%, 5,0%/3,5% and 4,7%/20,8% from the slabs which were respectively 6, 4 and 2 mm thick.

The range of adult *H. contortus* which failed to migrate from the 12 agar slabs was 0–11,6% (mean 6,3%); for the L4 the range was 0–22,7% (mean 5,0%).

The number of worms in each aliquot varied from a mean of 138 in the thickest slabs to 35 in the thinnest.

*Comment*

The results obtained with the different thicknesses of agar cannot be compared as there was a wide variation in the percentages of adults and larvae that failed to migrate and also in the total number of worms present in the agar slabs. However, it appears that migration was not affected by the thickness of the agar in the ranges that were tested. Poor results were apparently obtained with the *H. contortus* L4 in the case of the thin slabs, but there were very few worms in the agar and in each case where >20% larvae apparently failed to migrate, only 1 larva was recovered from each 20% aliquot of ingesta examined. A total count of larvae remaining in the agar would have given more accurate results.

### *IV. Migration after various time intervals between autopsy and the processing of the ingesta*

An important question is how long after collection and before being processed may worms be left at room temperature in ingesta without losing the ability to migrate.

*Method*

Sheep 5 and 6 were infested on 3 occasions with *H. contortus* and *T. colubriformis*, 42, 39 and 36 days before slaughter. In addition the 2 sheep were again infested with *H. contortus* 4 days before being killed.

At autopsy the worms from both sheep were pooled in physiological saline, mixed, and divided roughly into 6 aliquots. These were allowed to stand at room temperature for periods varying from 0,5–4,5 h before being subdivided into 3 portions each and gelled in agar (final concentration 0,65%). Subsequently the agar preparations were incubated in a waterbath for worm recovery.

The temperature of each aliquot of ingesta was recorded immediately before the ingesta were gelled in agar (Tables 4, 5 & 6).

Although *H. contortus* adults and L4 and *T. colubriformis* adults were mixed in the ingesta, each species and stage of development are listed separately in Tables 4, 5 and 6.

While total counts were made of the worms that migrated, only 20% of each intestinal residue was examined for worms which had failed to migrate out of the agar.

*Results*

The results are listed in Tables 4, 5 and 6.

The number of adult *H. contortus* that failed to migrate from the agar varied from 4,2% at 0,5 h–20,3% at 3,5 h. With 2 exceptions (one aliquot each at 1,0 h and 1,5 h) all the L4 *H. contortus* migrated from the agar. The numbers of adult *T. colubriformis* that remained in the agar varied from 0% (at 0,5 h and 1,0 h) to 15,6% at 4,5 h.

No larval *T. colubriformis* was recovered.

*Comment*

Although only a few aliquots were tested and very few L4 *H. contortus* were present, it appears that adult worms were more markedly affected by being left at room temperature than the larvae.

Fewer adults of both *H. contortus* and *T. colubriformis* migrated after standing at room temperature than when the ingesta were processed immediately after collection. On the other hand it must be borne in mind that relatively large variations in migration occurred in the other experiments (vide Tables 3, 7 & 9) where these are considered incidental. With *T. colubriformis* only small differences occurred between aliquots gelled 0,5 h–3,5 h (0%–5,0% migration failure) after autopsy.

More tests should be undertaken to confirm these results.

It must be noted that the concentration of agar used in this experiment (0,65%) is lower than the minimum optimal concentration used in Experiment II. This agar was a new batch, while that used in the other investigations described in this paper were from a batch which had been opened long before use.

### *V. Migration from agar without additives of adult worms belonging to 6 nematode species*

*Method*

Sheep 1 was infested as described in Experiment I. Sheep 7 and 8 were both infested with *Ostertagia circumcincta, Trichostrongylus colubriformis* and *Nematodirus spathiger* and Sheep 9 with *Strongyloides papillosus.*

The agar concentrations used and the ages of the worms at autopsy are listed in Table 7.

*Results* (Table 7)

With the exception of *N. spathiger* and *T. colubriformis*, from 100% (*O. circumcincta*)—85% (*S. papillosus*) migrated from the agar. In the first trial 50% *N. spathiger* and 32,6% *T. colubriformis* failed to migrate (Sheep 7) but in a subsequent trial only 2,5% and 4,5% respectively did not migrate (Sheep 8).

With the exception of 1 aliquot of *N. spathiger* which migrated slowly, the migration during the first 6 h of incubation ranged from 87,8% (*T. colubriformis*)—100,0% (*O. circumcincta* and *O. columbianum*) of those that did migrate.

*Comment*

The results varied with the experimental animals and the worm species. Nevertheless, the only worm species for which migration was poor was *S. papillosus*, 15% of which worms failed to migrate.

TABLE 7 Migration of adult worms from agar

| Sheep No. | Agar (%) | Species | Period of infestation (Days) | No. of worms recovered | | |
|---|---|---|---|---|---|---|
| | | | | 24 h incubation | 6–24 h incubation | In agar (%) |
| 1 | 0,80 | *H. contortus* | 35 | 255* | 2 | 3,7 |
| 1 | 0,80 | *O. columbianum* | 35 | 141 | 0 | 4,3 |
| 7 | 0,85 | *O. circumcincta* | 38 | 260 | 6 | 0,0 |
| 8 | 0,85 | *O. circumcincta* | 43 | 95 | 0 | 8,4 |
| 7 | 0,90 | *T. colubriformis* | 38 | 706 | 58 | 32,6 |
| 8 | 0,85 | *T. colubriformis* | 43 | 2 984 | 78 | 2,5 |
| 7 | 0,90 | *N. spathiger* | 38 | 192 | 60 | 50,0 |
| 8 | 0,85 | *N. spathiger* | 43 | 333 | 21 | 4,5 |
| 9 | 0,90 | *S. papillosus* | 31 | 213 | 9 | 15,0 |

* The mean of 2 results (Table 1)

TABLE 8 Migration of 4th stage larvae from agar

| Sheep | Worm species | Age of worms (days) | No. of worms recovered | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0,85% agar | | | 0,90% agar | | |
| | | | 24 h incubation | 6–24 h incubation | In agar (%) | 24 h incubation | 6–24 h incubation | In agar (%) |
| 7 | *O. circumcincta* | 9 | 811 | 47 | 27,0 | — | — | — |
| 8 | *O. circumcincta* | 12 | 2 640 | 17 | 0,0 | — | — | — |
| 10 | *H. contortus* | 7 | 441 | 34 | 4,8 | 675 | 45 | 9,5 |
| 10 | *C. ovina* | 7 | 237** | 3 | 0,9 | 23† | 3 | 0,0 |
| 11 | *G. pachyscelis* | 23 | 40 | 7 | 7,5 | 51 | 1 | 3,9 |
| 11 | *O. columbianum* | 23 | 59 | 1 | 3,4 | 21 | 0 | 0,0 |
| 12 | *O. columbianum* | 7 | — | — | — | 382 | 2 | 0,8 |

** Combined results of aliquots from both the small and the large intestinal ingesta
† Only the large intestinal ingesta were tested

The poor migration of *T. colubriformis* and *N. spathiger* from the same agar slab in one trial (Sheep 7) may possibly be ascribed to worm damage caused when the ingesta were pressed to remove excess water. In a subsequent trial (Sheep 8), when the ingesta were mixed with an equal volume of agar, only a small number of the worms failed to migrate. Larval *O. circumcincta* from Sheep 7 also migrated poorly (Experiment VI). Nevertheless, the migration of adult *O. circumcincta* from abomasal ingesta of this sheep was high (100%), despite similar preparation to the small intestinal ingesta.

The migration was slower in this experiment than in Experiment I and showed that incubation for at least 24 hours is essential. That experiment was the only one in which tap water alone was used for dissolving the agar, saline having been used in all the other trials.

### *VI. Migration of 4th stage larvae (L4)*

*Method*

Sheep 7 and 8 were infested with *O. circumcincta*, Sheep 10 with *H. contortus* and *Chabertia ovina*, Sheep 11 with *Gaigeria pachyscelis* and *O. columbianum*, and Sheep 12 with *O. columbianum*.

The ages of the worms at necropsy and the concentrations of agar tested are listed in Table 8.

*Results* (Table 8)

As in the case of adult worms, it was found necessary to incubate L4 for 24 h.

With one exception, namely *O. circumcincta* in Sheep 7, in which 27% of the larvae remained in the agar, the percentage of larvae which failed to migrate ranged from 9,5% (*H. contortus*)—0% (*O. circumcincta* from Sheep 8 and *C. ovina* and *O. columbianum* from Sheep 12).

The numbers of worms recovered from 0,85% agar and 0,90% agar were similar.

*Comment*

Migration of the various 4th stage larvae (L4) was similar to that of adults and larvae in Experiment III, V & VI in that poor migration was observed with *O. circumcincta* only in Sheep 7. In Sheep 8, however, satisfactory migration was obtained with much larger numbers of *O. circumcincta*.

### *VII. Migration of parasitic L3 from rumen ingesta*

*Method*

Sheep 13 was infested with 400 000 *H. contortus* L3, administered in 4 equal doses 22 h, 7 h, 4 h and 2 h before it was killed.

At autopsy aliquots of rumen ingesta were embedded in 0,85% agar and incubated for 24 h.

*Results*

The results are summarized in Table 9.

A mean of 7,5% of the larvae, with a range of 4,0%–12,7%, failed to migrate from the agar.

All but 1,5% of the larvae examined had exsheathed by the end of the incubation period.

TABLE 9 Migration of parasitic L3 *H. contortus*

| Aliquot No. | No. of worms | | |
|---|---|---|---|
| | Total No. | Failed to migrate | In agar (%) |
| 1 | 776 | 31 | 4,0 |
| 2 | 424 | 25 | 5,9 |
| 3 | 743 | 60 | 8,1 |
| 4 | 418 | 45 | 10,8 |
| 5 | 614 | 50 | 8,1 |
| 6 | 398 | 50 | 12,6 |
| 7 | 993 | 60 | 6,0 |
| 8 | 433 | 55 | 12,7 |
| 9 | 800 | 55 | 6,9 |
| 10 | 551 | 30 | 5,4 |
| 11 | 563 | 50 | 8,9 |
| 12 | 549 | 35 | 6,4 |
| Mean % in agar | — | — | 7,52 |

*Comment*

These results are very similar to those obtained with the other developmental stages of *H. contortus*. Recovery of larvae from the ruminal ingesta is usually a laborious process if one should, for instance, be studying the morphogenesis of worms, and it seems that this technique will facilitate their collection. However, incubation for 24 h may present the problem that the worms might develop further during this period, and it is uncertain how this would affect a comparison with development in the normal site. It is not known, for instance, whether some of the larvae exsheathed during the 24 h of incubation or had already exsheathed when the host was killed.

### *VIII. Attempts to stimulate migration*

In the previous trials it was only exceptionally possible to recover all the worms from the agar slabs by migration, as some usually remained in the agar slabs.

Van Wyk, Gerber & Van Aardt (1977) used formalin to stimulate the motility of nematode infective larvae while Jørgensen (1975) added bile to agar for recovering *Dictyocaulus* L3 from herbage. Both these substances were tested in this experiment in an attempt to stimulate greater migration from the agar suspension.

*Method*

Sheep 8 was infested with *O. circumcincta, T. colubriformis, N. spathiger* and *C. ovina,* and Sheep 15 with *H. contortus.* Sheep 9 and 12 were infested as described in Experiments V and VI.

Aliquots of ingesta from Sheep 8 and 15 were suspended either in agar containing from 0,10–0,25% formalin or in agar alone (Table 10). The ingesta of Sheep 9 and 12, on the other hand, were mixed with agar alone, with agar containing 1% bile or 0,05% formalin, and with agar containing both bile and formalin (Table 11).

The details of the agar concentrations, ages of the worms at necropsy and substances tested are listed in Tables 10 and 11.

TABLE 10 Effect of formalin on the migration of various nematodes

| Sheep | Worm species | Age of worms (days) | No. of worms recovered | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Agar** alone | | | Agar+formalin | | | |
| | | | 24 h incubation | 6–24 h incubation | In agar (%) | Formalin concentration (%) | 24 h incubation | 6–24 h incubation | In agar (%) |
| 8 | *O. circumcincta* adults | 43 | 95 | 0 | 8,4 | 0,05 | 113 | 0 | 7,1 |
| 8 | *O. circumcincta* L4 | 12 | 2 640 | 17 | 0,0 | 0,05 | 1 657 | 9 | 4,0 |
| 8 | *T. colubriformis* adults | 43 | 2 984 | 78 | 2,5 | 0,05 | 2 920 | 159 | 46,3 |
| 8 | *N. spathiger* adults | 43 | 333 | 21 | 4,5 | 0,05 | 443 | 44 | 42,0 |
| 8 | *C. ovina* adults | 43 | — | — | — | 0,05 | 475 | 37 | 48,4 |
| 15 | *H. contortus* L3 | 1 | 16 | 4† | 25,0 | 0,10 | 37 | 4 | 35,1 |
| 15 | *H. contortus* L3 | 1 | 16 | 4† | 25,0 | 0,25 | 35 | 0 | 34,3 |

** 0,85% for Sheep 8; 0,90% for Sheep 15
† The same result repeated as a control for each concentration of formalin tested

TABLE 11 The effect of bile and/or formalin on the migration of adult *S. papillosus* and L4 *O. columbianum*

| Sheep | Worm species | Age of worms (days) | Treatment | No. of worms recovered | | |
|---|---|---|---|---|---|---|
| | | | | 24 h incubation | 6–24 h incubation | In agar (%) |
| 9 | *S. papillosus* | 31 | Agar* without other additives | 213 | 9 | 15,0 |
| | | | Agar and bile† | 368 | 7 | 8,7 |
| | | | Agar and 0,05% formalin | 373 | 24 | 64,9 |
| | | | Agar and bile†+0,05% formalin | 498 | 42 | 25,1 |
| 12 | *O. columbianum* | 7 | Agar without additives | 382 | 2 | 0,8 |
| | | | Agar+bile† | 511 | 6 | 0,2 |

* 0,9% agar
† Roughly 1% concentration

TABLE 12 Migration of larvae in the histotrophic phase

| Sheep | Worm species | No. of worms in aliquot | | In agar (%) |
|---|---|---|---|---|
| | | Without agar | Agar | |
| 12 | *O. columbianum* L4 (Aliquot 1) | 553 | 529 | 3,6 |
| 12 | *O. columbianum* L4 (Aliquot 2) | — | 432 | 2,6 |
| 14 | *O. columbianum* L3 | 39 | 24 | 9,1 |
| 15 | *H. contortus* L3 | 16 | 32 | 18,8 |

*Results*

The percentage of worms that failed to migrate from the agar alone ranged from 0–25%; the corresponding range for agar plus low concentrations of formalin was 4–64,9% (Tables 10 & 11).

When 1% bile was added to the agar, 8,7% adult *S. papillosus* and 0,2% *O. columbianum* failed to migrate, and, when it was omitted, 15,0% *S. papillosus* and 0,8% *O. columbianum* did not migrate. In agar plus bile and formalin, a lower percentage of *S. papillosus* adults remained in the gel than in agar plus formalin alone, namely 25,1% and 64,9% respectively.

*Comment*

It is clear that further tests with a wider range of concentrations of these additives will have to be carried out before their value can be established, but it seems that the addition of bile probably facilitates the migration of the worms in suspension. Formalin, on the other hand, appears to be inhibitory as in only one instance (*O. circumcincta* adults) did more worms migrate from the agar plus formalin than from agar alone.

No conclusions could be arrived at about the effects of additives on the migration of parasitic L3 *H. contortus* from the agar since insufficient of these larvae were present in the aliquots tested.

### *IX. Migration of larvae in the histotrophic phase*

Pepsin/HCl digestion is usually used for recovering larvae in the histotrophic phase (Reinecke, 1973), but, since detection of the larvae in the digest requires a great deal of microscopic work, it was decided to test agar migration to facilitate recovery.

*Method*

Sheep 12 and 14 were infested with L3 *O. columbianum* 7 days and 2 days respectively before necropsy and Sheep 15 with *H. contortus*, 24 h before slaughter (Table 12).

At necropsy the abomasal (Sheep 15) or intestinal mucosa (Sheep 12 and 14) was stripped, blended (Reinecke, 1973) and divided into either 3 (Sheep 12) or 2 aliquots (Sheep 14 and 15) of equal mass. One aliquot from each sheep was digested with pepsin/HCl while the rest were gelled in agar slabs and incubated at 40 °C.

After incubation the agar was melted and the residue examined microscopically for worms that had failed to migrate. Thereafter the residue was digested with pepsin/HCl and finally re-examined *in toto* microscopically.

*Results*

A range of 2,6%–18,8% of the larvae failed to migrate from the agar (Table 12).

The numbers of larvae recovered from 2 aliquots of gelled mucosa after migration and digestion of the residue were similar to those recovered from a duplicate aliquot treated by digestion alone (529 and 432 compared with 553; Table 12).

*Comment*

Since the larvae remaining in the agar might possibly be more difficult to detect than in aliquots digested with pepsin/HCl, we examined the residue after melting the agar and again after digestion. Furthermore, we considered the possibility that the larvae remaining in the residue might have been damaged during the melting process and subsequently been digested, so we treated and examined a duplicate aliquot in the conventional way.

Since the total numbers of larvae recovered from the gelled aliquots were similar to those of the aliquot treated conventionally, it is probable that the results give a reasonably accurate estimate of the total worms that migrated or failed to migrate.

Although only very small numbers of parasitic L3 *O. columbianum* and *H. contortus* were present, their migration was similar to that of older larvae.

The sheep used in this trial had been reared under wormfree conditions and were killed shortly after infestation with fresh, unrefrigerated, laboratory-produced infective larvae. It is unlikely, therefore, that any of these larvae were hypobiotic and that their further migration and development would have been delayed, had the sheep not been killed. Whether hypobiotic larvae, which have been delayed in the histotrophic phase for long periods (e.g. months), will be more lethargic and hence be less inclined to migrate than the larvae in this trial is an aspect which should be investigated.

## Discussion and Conclusions

The use of a newly-opened batch of agar in Experiment IV gave better gel strengths at similar agar concentrations than the old batch which had been opened long before use for worm recoveries and was used in all the other experiments. Furthermore, when the new batch was retested later (Gerber, unpublished data, 1977), it gave similar results to the old batch. It would appear, therefore, that different batches of agar, after being opened should be tested for gel strength before use and retested if not used within a short time.

When the ingesta were processed immediately after collection at autopsy, the mean percentage migration of adult worms and larvae from agar gel without the

addition of bile or formalin was 93,6%. Worms were almost free from particulate contamination and hence were easily counted.

Although this mean percentage migration is much higher than the mean of 65% for migration of infective larvae of *Dictyocaulus* spp. larvae from 1,3% agar (Jørgensen, 1975), variations between aliquots were sometimes inexplicably large (vide Tables 3, 7 & 9). Possible reasons for this (for example, variations in the concentrations of ingesta in the gel) should be investigated. Further possible investigation would profitably include the search for better stimulants or the introduction of a further step (for example, differential sedimentation) after incubation of the agar slabs. Naturally, the recovery of worms from bovine and other ruminant ingesta should also be tested.

At this stage the method is probably of value for the qualitative recovery of worms (for example, for taxonomic work), but is probably unsatisfactory for quantitative worm recovery. If the total intestinal ingesta must be examined in any case then the time saved may be offset by the added expense of applying the agar technique.

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