AN IMPROVED TECHNIQUE FOR THE INCUBATION OF NEMATODE EGGS IN FAECES

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Summary

The culturing of faecal samples contaminated with nematode eggs has been the regular technique for producing large numbers of nematode larvae for larval studies. However, methods already in use were found unsatisfactory for the in vitro studies of the factors affecting exsheathment of the infective larvae of the sheep stomach worm, Haemonchus contortus, one of the most important parasites of sheep and other ruminants.

An improved technique for the incubation of nematode eggs in faeces is described. The new technique eliminates the disadvantages of older methods, such as the time-consuming weighing and measurement of faecal samples and liquid media. The new technique also produces large numbers of vigorous larvae, in clean aqueous suspension. The larvae live longer, and respond consistently to different treatments. The method is simple, time-saving and yields consistent results.

Materials and Methods

Fresh faeces with at least 5,000 eggs per gram of faeces (EPG) were thoroughly commuted in 0.1 per cent sodium carbonate solution which was on tap at a convenient height from the working table (see fig. 1). Enough solution was added to the faecal sample to produce a smooth, viscous mixture. The viscous faecal mixture was then mixed with pulverized sterile medium to produce a firm consistency just wet to the touch. The sterile medium had been previously prepared from sun-dried sheep faeces, ground in a coffee grinder into fine powder, and autoclaved for 30 minutes at a pressure of about 15 lbs.

Several Syracuse dishes were filled with the mixture and the dishes wiped clean around the rim (see fig. 2 stage 1). The charged Syracuse dishes were next placed in deep petri dishes, and the cultures arranged on shelves in an incubator set at a constant temperature of 21.5°C. By means of a long delivery tube leading from a reservoir (which was set at a convenient height), each petri dish was filled with triple distilled water to about two-thirds the height of the Syracuse dish (see fig. 2 stage 1). This height of the water surrounding the Syracuse dish was easily accessible to the migrating larvae but low enough...
to avoid flooding the culture and contaminating the water. Finally, each petri dish was covered with the lid, and the cultures left in the incubator (21.5°C) for six days. (Plate 1 shows cultures arranged in an incubator).

At the end of the six days, the cultures were transferred into a second incubator at a constant temperature of 28.5°C and left for two more days. During this period, the infective larvae migrated from the faecal culture into the surrounding water.

To recover the larvae, the water surrounding the Syracuse dishes and containing the larvae, was poured into a beaker as illustrated in stage 3 of Fig. 2. The Syracuse dish was then removed and the petri dish rinsed with more distilled water to recover the remaining larvae. The water containing the larvae (described as “the larval suspension”) was poured onto 8-inch milk filters in a pie-pan modification of the Baermann funnel*, adapted by the author for the recovery of larvae from aqueous suspensions.

Since larvae stored in coolers at 40°F were found to be sluggish and short-lived, cultures were kept at room temperature until required. To remove dead larvae from the cultures from time to time, cultures were poured onto fresh milk filters in the pie pans once a week, and the living larvae permitted to migrate through the filters into clean water while the dead larvae stayed behind on the filters. Cultures three or more weeks old were discarded.

The number of larvae in a stock culture was estimated by counting under low power, the larvae present in randomly drawn aliquot parts of larval suspension. The stock cultures were labelled to indicate the number of larvae per millilitre of suspension.

Discussion and Conclusion

Studies on factors influencing the exsheathing mechanism of the infective larvae of *Haemonchus contortus* required certain specific conditions to produce meaningful results. One important condition was that the watery medium in which the larvae were to be studied had to be absolutely clean. Methods of larval culturing used before the present system was evolved, often produced cultures which were contaminated with faecal debris and colouring inorganic matter. In some instances—and this often happened in about 60 per cent to 70 per cent of the cultures—the water surrounding the cultures ran into the faecal sample and caused the latter to putrefy. The contaminated watery medium adversely affected the longevity of the larvae, many of which died even before the cultures were harvested. The remaining living larvae were often lethargic, and responded feebly or inconsistently to various treatments.

For reasons which are not yet understood, the old culture techniques produced fewer larvae. This was a definite drawback on the study, especially when large numbers of larvae were required.

The improved technique had several advantages. It did not involve any weighing of faecal samples or the measurement of definite volumes of liquids. This was a great saving in time and material. Large quantities of vigorous larvae were harvested at the end of the incubation period. The aqueous medium in which the larvae were collected was invariably clean, because handling had been reduced to the minimum, and done only at a stage when there was no risk of spilling the water on the faecal samples.

In previous techniques, the petri dishes in which the faecal cultures were placed, were charged with water on the working table and then transferred to the incubators. During this transfer, the water often spilled over the faecal mass in the Syracuse dishes, so that at the end of the incubation period the culture had already started decomposing, creating an anaerobic micro-climate in the particular dish with deleterious effects on the longevity of the larvae. Placing the dishes in the incubator before charging with distilled water through a delivery tube that could reach far to the rear of the incubator, completely eliminated this source of loss which often followed an otherwise successful incubation. The larvae, recovered from the aqueous

medium by the modified pie-pan type of Baermann funnel (Baermann, 1917) remained vigorous for long periods at room temperature, and responded consistently to the same treatment in the study.

With the apparatus assembled as shown in Fig. 2 the procedure for setting up the cultures followed a simple stage-by-stage pattern, convenient even when very large cultures were to be set up.

\[ A = 0.1\% \text{ Sodium carbonate solution} \]
\[ B = \text{Triple distilled water} \]
\[ C = \text{Sterile medium} \]
\[ D = \text{Syracuse dishes} \]
\[ E = \text{Petri dishes} \]
\[ F = \text{Incubators} \]

**FIG 1 ASSEMBLAGE OF APPARATUS FOR FAECAL CULTURES.**
1. Fresh faeces + 0.1% Sodium carbonate solution + Sterile medium

Syracuse dish

2. Petri dish

Triple distilled water

Recovering larvae in distilled water

3. Larvae in clean distilled water

FIG 2 STAGES IN THE TECHNIQUE OF FAECAL CULTURING.
Plate 1

Cultures arranged in an incubator.
References


