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Brief Communication

Comparison of Two Techniques Used for Quantification of Ovine Gastrointestinal Nematode Larvae in Herbage

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The assessment of populations of free living stages of gastrointestinal nematodes is important to predict uptake in livestock in relation to time and thus potential risk periods for trichostrongylosis. Several methods are currently used to assess the density of infective larvae (L3) of parasitic nematodes in herbage samples. Larval suspensions obtained by baermannization or similar sedimentation methods often contain considerable amounts of debris that make microscopic examination tedious and time consuming. To overcome this, *Jørgensen* (1975) developed the agar gel migration technique to obtain a suspension of L3 of *Dictyocaulus viviparus* free of debris. The migration from the agar film was later evaluated and found suitable for isolation of gastrointestinal nematodes of cattle and sheep (*Mwegoha & Jørgensen* 1977). The present study compares a Baermann method (*Persson* 1974) with the agar gel migration method for the isolation of ovine gastrointestinal nematode larvae from naturally contaminated herbage.

Forty-eight herbage samples were collected over the course of an entire grazing season from 4 different paddocks permanently grazed by sheep that were excreting eggs of *Nematodirus, Ostertagia* and *Trichostrongylus* spp. The paddocks were traversed in a W-shape stopping every 5 m and 3 small wisps of herbage were picked and placed in separate plastic bags randomly. The 3 samples per paddock were weighed (200-500 g) and randomly allocated to the following methods: one sample was processed by the Baermann method, using a metal sieve (∅ 200 μm) suspended in water (*Persson* 1974), and the 2 remaining samples were processed by the agar gel migration technique modified after *Jørgensen* (1975) and *Mwegoha & Jørgensen* (1977) by omitting bile in the agar. Briefly, the herbage was soaked overnight in water, washed in a concrete mixer and sieved through a cloth of 31 μm. The debris content was embedded in agar on a cloth, submerged in water at room temperature overnight, whereafter the emigrated larvae were enumerated. The herbage of the 2 agar processed samples was reprocessed immeadiately by the Baermann technique in order to recover any residual larvae. These residual numbers added to the number of L3 recovered by the agar gel migration method were used as an estimate of the total number of larvae in the sample, even though more larvae were likely to remain in the sample. Recovered larvae were stained with iodine

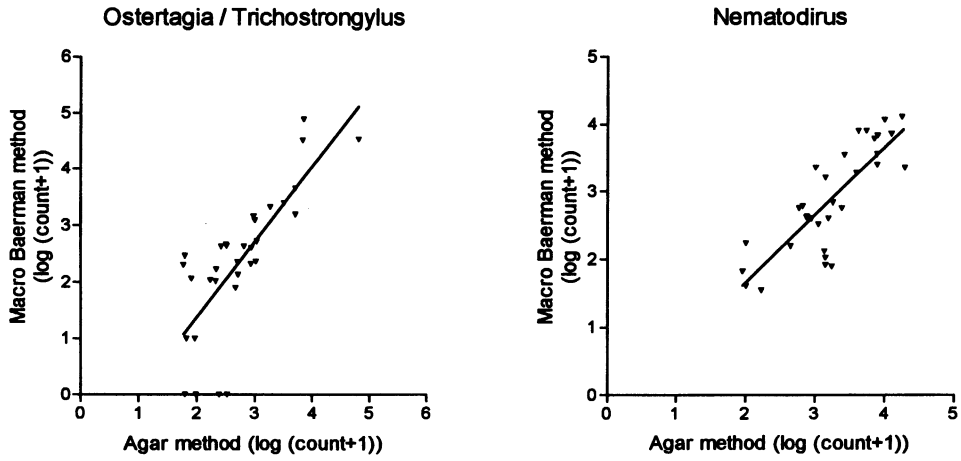

Figure 1. Comparison of the recovery of agar gel migration and macrobaermann methods of *Ostertagia/Trichostrongylus* (left) and *Nematodirus* (right) spp. infective larvae from pastures using simple regression line on log transformed counts.

(*Jørgensen* 1975) and counted under microscope. The genera of parasitic larvae were determined on morphometric details, grouping *Ostertagia* spp. and *Trichostrongylus* spp. together. The 2 replicates of the agar gel migration method were averaged. Herbage dry weight was determined after one month of air drying at room temperature. The data was analyzed by paired t-test and regression analysis on log transformed counts.

The agar gel migration technique showed a higher recovery of L3 of both *Ostertagia/Trichostrongylus* and *Nematodirus* spp. as compared to the Baermann technique ($p<0.05$ and $p<0.001$, respectively by paired t-test). Agar gel migration showed the highest recovery of *Ostertagia/Trichostrongylus* L3 in 21 of the 32 samples, and of *Nematodirus* spp. in 25 samples. There was a close correlation between the log-transformed counts of the 2 methods ($r = 0.77$ and $r = 0.82$, respectively) and the slopes of regression lines for both genera were not significantly different from 1 (Fig. 1). However, more *Ostertagia/Trichostrongylus* spp. larvae were recovered by agar gel migration technique at low levels of pasture contamination, and in 4 out of 32 samples, L3 was only recovered by the agar gel migration method. The recovery of the agar gel migration method expressed as a percentage of estimated total larvae recovered was in most cases above 65% (lower 95% confidence limits) for *Ostertagia/Trichostrongylus* spp. and above 80% for Nematodirus spp. (Fig. 2). However, on 7 occasions the recovery rate of *Ostertagia/Trichostrongylus* was less than 50%. This was not related to the level of pasture contamination.

The study confirmed observations made by *Steffan* (1993) that the recovery of L3 was similar or better in agar-gel migration method compared to a modified Baermann method. The samples processed by agar-gel migration method were clean and hence, the larvae were easily detected. A lot of debris made the microscopic examination of the sediment of the baermannization tedious and some of the larvae may have been missed. Also sample sizes larger than 200 g have shown reduced recovery rates

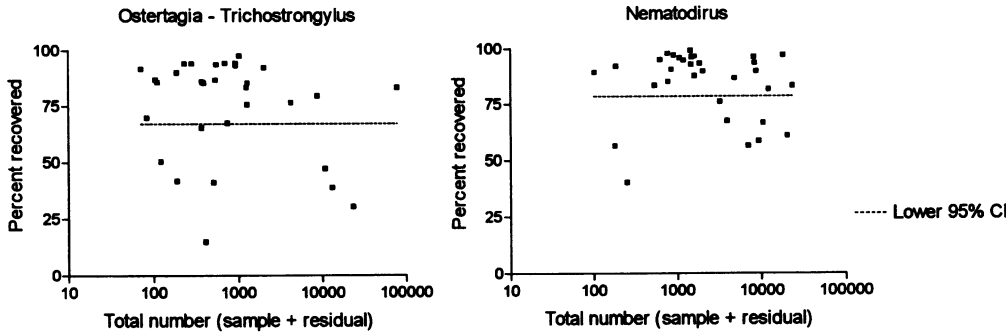

Figure 2. Number of infective larvae recovered by the agar gel migration method in per cent of total number of infective larvae recovered (sample+residual) for *Ostertagia/Trichostrongylus* spp. (left) and *Nematodirus* spp. (right). Horizontal dashed lines indicate lower 95% confidence limits of the recovery rates.

of the Baermann method (*Persson* 1974). Absolute recovery rates of larvae in herbage cannot be obtained by any method. In the present study the recovery rate was probably overestimated as total larvae, as defined here, was likely to be an underestimation of the absolute number. Previous experiments on recovery rates have been based on addition of cultured L3 to parasite-free herbage, and rates of 58%-77% and 30%-60% have been reported for the Baermann method (*Persson* 1974, *Henriksen* 1982) and of 60%-69% for the agar bile migration method (*Eysker & Kooyman* 1993). However, addition of larvae may also lead to an overestimation of recovery rates as larvae are not attached to the herbage as in naturally infected herbage samples and may thus be more easily recovered. The ability of the larvae to detach from the vegetation may depend on several factors, including genus and age of larvae, and type and stage of growth of herbage. This could be one of the reasons for the high number of residual larvae after the agar method observed in a few instances. A certain, unknown loss during the agar embedding step is likely to take place. To investigate if it was possible to substitute the agar separation step of the modified agar method with a simple baermanization of the debris, 14 samples of debris with larvae following the washing procedure (approx. 100 ml) were divided into 2 equal portions and processed either by agar embedding as previous samples or by simple baermannization. The baermannization was carried out using 2 pieces of gauze, 2 pieces of lens cleaning paper (Japico Drissler, D-63114 Dietzenbach) and a 500 ml plastic jar (Fig. 3). The bottom of the plastic jar was removed. In between 2 pieces of gauze, 2 pieces of lens tissue paper were placed, put on the mouth of the plastic jar (diameter 5.5 cm) and tied tightly around the neck of the jar. The jar with filter was inverted and put into the funnel of a Baermann's apparatus and water added until the filters were submerged. Samples were poured gently into the inverted jar on the side walls and left overnight at room temperature. Approx. 10 ml of fluid was withdrawn, centrifuged and the sediment examined. In 11 out of 14 instances, more larvae were recovered by the tested baermannization compared to the agar migration (paired t-test: $p<0.001$). As the number of L3 increased, the difference gradually became smaller.

This preliminary testing of a new procedure, bearing resemblance to the technique of *Gettinby et al.* (1985), revealed that the agar migra-

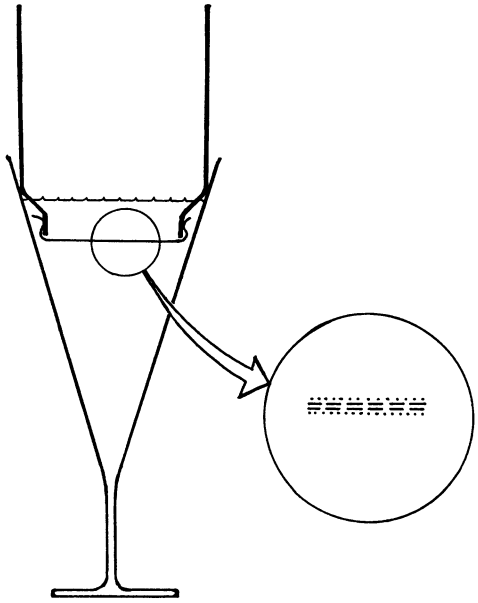

Figure 3. The set-up for Baemanization of the L3 from debris. The baermannization was carried out using 2 pieces of gauze and 2 pieces of lens cleaning paper on the mouth of a plastic jar.

tion step can be replaced by a simpler baermannization without any loss in recovery rates. More larvae may even be recovered, perhaps due to trapping of some physically weak larvae in the solidified agar. *Mwegoha & Jørgensen* (1977) estimated a retention of larvae in the agar of approx. 30%. In our study, both methods yielded similar clean suspensions of larvae. However, the method combining soaking, vigorous washing, sieving and baermannization may be most suitable in field work, especially in places where resources are scarce, as it is less costly and less time consuming. When interpreting pasture larval counts one should always consider variable recovery rates and repeated measurements should be advocated.

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