An Evaluation of the Baermann Technic using Infective Larvae of *Haemonchus contortus*[1]

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ABSTRACT: An evaluation of the modified Baermann technic, using infective *Haemonchus contortus* larvae, was made to determine the effects of time, type of filter, temperature, vehicle (solution), illumination, size of funnels, amount and length of grass, and weight and type of soil on the numbers of larvae recovered. A higher percentage of larvae was retrieved from funnels in which cheesecloth was used as a filter than from those containing cellulose tissue. Greater percentages of larvae were recovered at 4, 10, 20 and 25 C than at 30–50 C. Tap water, 0.9% NaCl solution, mammalian Ringer's solution and 0.2% HCl were equally effective as vehicles for baermannization, but a non-ionic and two anionic detergents were less satisfactory and at some concentrations were toxic to the larvae. There was no significant difference in larval recovery in the light or dark. The greater the diameter of funnel used for baermannization, the fewer larvae were recovered. The greater the weight of grass placed in the funnels, the lower was the recovery rate. Retrieval of larvae was better from sand than from silty clay loam soil, and better from silty clay loam than from clay loam.

A method to extract hookworm larvae from soil was described by Baermann (1917a). A modified Baermann apparatus was described by Cort et al. (1922) and is widely used by parasitologists to recover nematodes from soil, grass and feces. Dinaburg (1942) studied the efficiency of this technic and the variation in results that are obtained when using it. Because of the routine use of the modified Baermann technic in our laboratory to recover infective larvae of *Haemonchus contortus* and other trichostrongylids from soil, grass and feces, a detailed evaluation of this method was needed.

Materials and Methods

Fecal pellets containing 5,000–25,000 *Haemonchus contortus* eggs per gram were collected from monospecifically infected sheep. The pellets were incubated at 30 C for 5–7 days, at which time infective third stage larvae were present.

To determine the factors which affect the efficiency of the Baermann technic, the following variables were evaluated: (1) time of baermannization, (2) type of filter, (3) temperature, (4) vehicle (solution) used, (5) illumination, (6) size of funnel, (7) amount and length of grass, and (8) soil type and amount of soil.

The baermannization apparatus that was used as a standard for comparison consisted of a glass funnel 10 cm in diameter with a capacity of approximately 175 ml. A piece of 6 mm mesh galvanized wire screen was placed in the funnel approximately 4 cm from its top. A single layer of cheesecloth was placed over the wire screen, and the funnels were filled with warm tap water (about 25 C) to a level about 2 cm above the wire mesh. The fecal pellets were then placed in the water, which was of sufficient depth to cover them. As samples were withdrawn from the funnels water was added to restore the approximate initial level.

To determine the total number of larvae remaining in the Baermann apparatus after the samples had been withdrawn, the remaining fluid was removed, the funnel was washed with water, the tissue or cheesecloth was shaken in tap water to remove larvae, and the pellets were crushed and mixed in water. Aliquots of all these solutions were examined and the live (moving) larvae present were counted.

Experiment I, which was to determine the effects of time and the type of filter through which the larvae had to pass, was divided into six parts. Ten funnels, each containing 10 g of feces were used for each part. Five ml samples were withdrawn hourly for 8 hr and then at 12 hr; 24 hr after beginning the ex-

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periment, a final 15 ml sample was taken. In part one of the experiment, whole pellets and a single layer of cheesecloth were used; in part two, whole pellets and a single layer of cellulose tissue ("Kimwipes") were used. In part three, pellets were crushed and a single layer of cheesecloth was used. Part four was the same as part three except that a single layer of tissue was placed in the funnels. In part five crushed pellets were autoclaved, allowed to cool and a known number of larvae in 5 ml of tap water were mixed with the feces. The samples were covered and placed in the dark for 16 hr to allow uniform mixing of the larvae, after which they were baermannized using a single layer of cheesecloth. In part six, the Baermann apparatus, containing a single layer of cheesecloth and whole pellets, was shaken at 1 oscillation/sec throughout the experiment.

Experiment II was designed to determine the effect of temperature on baermannization. Five 10 cm funnels, each containing 10 g of feces, were placed in incubators at the following temperatures: 4, 10, 20, 25, 30, 35, 40, 45, and 50 C. Five ml samples were taken from each funnel at 2 and 4 hr and a 15 ml sample was removed 6 hr after beginning the experiment.

Experiment III was identical to part one of experiment I, i.e., whole pellets and a single layer of cheesecloth were used; however, samples were withdrawn at 2, 4 and 6 hr, and no wire screens were used in the funnels. The following solutions, in addition to tap water, were tested: 0.9% NaCl, mammalian Ringer's solution, 2.0% HCl, 0.2% Tergitol Wetting Agent No. 7, 0.2% Tergitol Penetrant 4, and 0.2% Tween-80.

Experiment IV was designed to determine the effect of illumination on baermannization. Five funnels were placed in the dark and five funnels were placed in a well lighted room at about 25 C. Whole pellets and a single layer of cheesecloth were used, and samples were withdrawn at 2, 4 and 6 hr.

Experiment V was similar to experiment I, part one except that different sizes of funnels were used. Five funnels of the following diameters and capacities were used for each part of the experiment; 7 cm, 80 ml; 10 cm, 175 ml; 13 cm, 350 ml; 15 cm, 650 ml; 25 cm, 3,700 ml; and 30 cm, 8,000 ml. All of the funnels were made of glass, except that the 25 cm funnels were plastic, and the 30 cm funnels were galvanized metal. Screens were of sufficient size so that they were situated approximately 4–6 cm from the top of the funnel. The mean total number of larvae remaining in the 10 cm funnels were used in computing the percentage recovery from the larger funnels. Samples were taken at 2, 4, 6 and 24 hr.

Experiment VI was conducted to determine the effects of the amount and length of grass on the efficiency of the technic. Because the grass samples we examine from pastures often contain dehydrated larvae, larvae were desiccated on grass for this experiment. Grass was cut from a Kentucky bluegrass pasture, washed in tap water and excess water was evaporated at room temperature. In part one of the experiment, grass approximately 2.5 cm long was used. Five samples each of grass weighing 5, 10. 20, 50, 100 and 150 g were mixed with known numbers of larvae. Each sample was then desiccated at 30 C and 70% relative humidity for 24 hr. After desiccation the containers in which drying took place were examined to determine the number of larvae that did not remain on the grass. At the same time that the grass samples were dried, larvae were desiccated in a Petri dish. This sample was rehydrated to determine the number of larvae that survived desiccation. The 5, 10 and 20 g samples were baermannized in 15 cm funnels, and the 50, 100, and 150 g samples in 30 cm funnels. Part two of the experiment was identical to part one, except that grass approximately 8.0 cm long was used. Samples were taken at 2, 4, 6 and 24 hr. All samples were placed in 2% HCl before counting to kill free-living nematodes that might have been on the grass.

In experiment VII the types and amounts of soil used for baermannization were evaluated. Three soil types (sandy, silty clay loam and clay loam) were used. Soil textural composition followed the particle class limits and basic soil textural classes defined by the Soil Survey Staff (1951). The content of clay and silt size particles was determined by the hydrometer method of Bouyoucos (1951). Particle dispersion was obtained by using sodium hexametaphosphate (Calgon) as a dispersing agent and a reciprocating shaker at 180 strokes/min. Soil pH was determined with a Leeds and Northrup glass electrode using a

Table 1. Characteristics of soil samples used for baermannization experiments.

Textural Class Name	% Sand 2.0–0.05 mm	% Silt 0.05–0.002 mm	% Clay < 0.002 mm	pH
Sand	93.6	1.2	5.2	7.3
Clay Loam	29.7	42.1	28.2	7.5
Silty Clay Loam	10.9	55.4	33.7	5.9

1 : 1 soil-water solution. All determinations were made in duplicate. Mean values are given in Table 1. Known numbers of larvae were mixed with 10 samples of each soil type of the following weights: 20, 40, 60, 80 and 100 g and placed in 10 cm funnels. A single layer of cellulose tissue was used for all parts of the experiment and samples were withdrawn at 2, 4, 6 and 24 hr.

In addition to the baermannization experiments, the effects of diluting larval suspensions on the accuracy of the counts were determined. When more than 200 larvae/ml were present, accurate counting was difficult because of the close proximity and activity of the larvae; it was also time-consuming. Samples containing approximately 100,000, 50,000, 25,000, 10,000 and 5,000 larvae were diluted to 500, 250, 125, 50 and 25 times their original volume, respectively, and 50 one ml samples were withdrawn with a pipette from each after bubbling air through the solution to mix the larvae.

Results

Experiment I:

Effects of time and type of filter

Figure 1 illustrates the effects of the use of cheesecloth and cellulose tissue on the recovery of larvae from crushed and whole pellets. Because a higher percentage of larvae was obtained using cheesecloth, this material was used for the other experiments. The following mean percentages (of the total number originally in the pellets) of larvae were recovered after 24 hr: whole pellets and cheesecloth, 87%; whole pellets and cellulose tissue, 67%; crushed pellets and cheesecloth, 85%; crushed pellets and tissue, 73%; larvae added to crushed pellets, 93%; and whole pellets and cheesecloth, sample shaken, 90%. The results

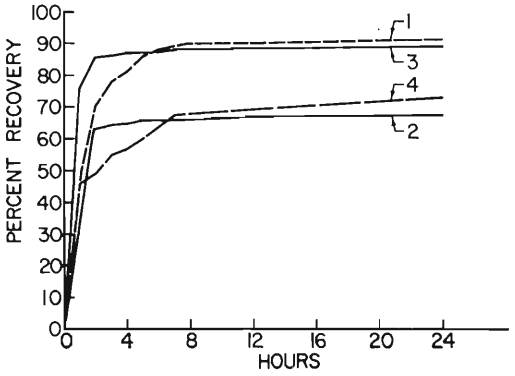

Figure 1. Percentage of larval recovery when cheesecloth or tissue was used with whole or crushed pellets: 1. Whole pellets, cheesecloth. 2. Whole pellets, cellulose tissue. 3. Crushed pellets, cheesecloth. 4. Crushed pellets, cellulose tissue.

of this experiment revealed that relatively few larvae were recovered after 6 hr. For this reason, in the other experiments samples were taken at 2 hr intervals up to 6 hr and in some experiments a final sample was taken 24 hr after the experiment began.

Experiment II: Effect of temperature

The effects of different temperatures on the percentages of larvae recovered are given in Figure 2. After 6 hr the following mean percentages of larvae were recovered: 4 C, 59%; 10 C, 64%; 20 C, 75%; 25 C, 63%; 30 C, 38%; 35 C, 39%; 40 C, 13%; 45 C, 5%; and 50 C,

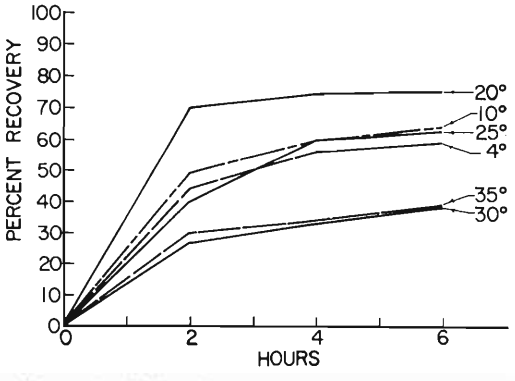

Figure 2. Effect of temperature on percentage of larval recovery.

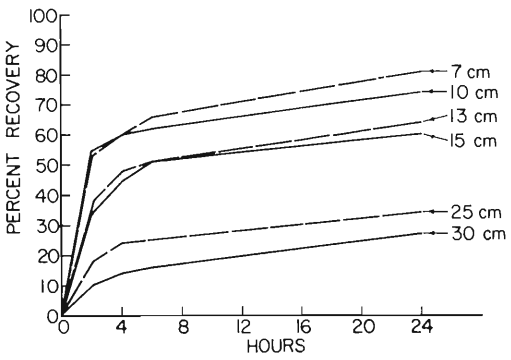

Figure 3. Effect of funnel diameter on percentage of larval recovery.

5%. At temperatures above 40 C most of the larvae were dead after 2 hr.

Experiment III: Effect of vehicle used

There were no significant differences in percentages of larvae recovered when water, salt or acid solutions were used for baermannization, but lower numbers were obtained when detergents were used. After 6 hr the following mean percentages of larvae were recovered: tap water, 83%; 0.9% NaCl, 76%; Ringer's solution, 96%; 2.0% HCl, 88%; 0.2% Tergitol Wetting Agent No. 7, 57%; 0.2% Tergitol Penetrant 4, 59%; and 0.2% Tween-80, 49%.

Experiment IV: Effect of illumination

This experiment revealed that there was no significant difference in percentages of larvae recovered when the funnels were placed in a well lighted room or in the dark. After 6 hr the mean percentages of recovery from the funnels placed in the dark and light were 84% and 86%, respectively.

Table 2. Percentages of recovery after 24 hr of baermannization of larvae desiccated on grass.

Weight of Sample (g)	% Recovery	
	2.5 cm Grass	8.0 cm Grass
5	36	28
10	28	32
20	19	33
50	19	7
100	19	7
150	4	4

Experiment V: Effect of size of funnel

The size of the funnel proved to be an important factor in determining the percentage of larvae recovered. The percentage decreased progressively as the funnel diameter increased (Fig. 3). After 24 hr the following mean percentages of larvae were recovered: 7 cm funnel, 83%; 10 cm, 76%; 13 cm, 68%; 15 cm, 65%; 25 cm, 47%; and 30 cm, 27%. The correlation coefficient for the percentage of larvae recovered from the above series after 24 hr was –0.99, which indicates a nearly perfect linear correlation.

Experiment VI: Effect of amount and length of grass

The results of parts one and two of experiment VI are given in Table 2. The values given are calculated as the mean percentages of larvae surviving desiccation in the controls placed in petri dishes.

Experiment VII: Effect of soil type and amount of soil

The results for this experiment are given in Table 3 and indicate that larger percentages of larvae were recovered from sandy and clay loam soils than from silty clay loam soil. A

Table 3. Percentages of larvae recovered from various weights of different soil types after 2, 4, 6 and 24 hr of baermannization.

	Sample Weight in Grams														
	Sand					Clay Loam					Silty Clay Loam				
Hours	20 g	40 g	60 g	80 g	100 g	20 g	40 g	60 g	80 g	100 g	20 g	40 g	60 g	80 g	100 g
2	42	34	21	22	15	33	21	14	14	10	8	9	8	7	7
4	47	53	31	27	19	39	26	22	18	13	14	13	9	9	10
6	52	58	41	29	22	43	29	26	20	16	18	16	10	11	11
24	66	63	51	37	26	45	32	29	23	17	21	18	11	11	11

higher mean percentage of recovery was obtained when small soil samples were used. Recovery from 20 or 40 g soil samples was generally two to three times greater than from 80 or 100 g samples.

In comparing the accuracy of the aliquot method of counting larvae, we found that when approximately 100,000, 50,000, 25,000, 10,000 and 5,000 larvae were diluted to 500, 250, 125, 50 and 25 times their original volume, respectively, the standard deviations for the numbers present were similar. The standard deviations for the above diluted volumes were 20.6, 24.0, 17.0, 18.9 and 19.6, respectively. However, the standard deviations for the calculated numbers of larvae present were: 10,312 for samples containing approximately 100,000 larvae, 6,015 for 50,000, 2,131 for 25,000, 950 for 10,000 and 495 for samples with about 5,000 larvae.

Discussion

Although higher percentages of larvae were recovered from funnels containing cheesecloth than from those containing cellulose tissue, large particles of debris passed through the cheesecloth and settled to the bottom of the funnel. Such debris did not pass through the cellulose tissues. Cort et al. (1926) used one or two layers of cloth in funnels and obtained slightly better results when only one layer was used. Tobar Jiménez (1963) evaluated five methods of recovering three genera of eelworms from soil samples. The modified cotton-wool method (Tobar Jiménez, 1962) and the Oosterbrink method, which used a double layer of milk filter pads through which the nematodes had to pass, gave significantly lower returns of the small nematode *Paratylenchus* spp. than the Seinhorst 2-flask method (Seinhorst, 1956) and the Seinhorst mistifier (Seinhorst, 1950), which did not have filters. When recovery of larger nematodes (*Helicotylenchus* and *Tylenchus*) was attempted, better results were obtained from the apparatuses which had filters. Kauzal (1940) used the Baermann apparatus; with a sieve only he recovered 58% of *H. contortus* and *Trichostrongylus* spp. larvae which had been placed in soil plots, but with gauze in the funnel, he recovered only 37% of the larvae.

Crushing the pellets did not increase the numbers of larvae recovered in our study and baermannized samples from crushed pellets were so turbid that the samples had to be centrifuged and washed before the larvae could be easily counted.

The relationship between the length of time samples are baermannized and the recovery of nematodes has not been adequately studied. Cort et al. (1922) reported that the moisture content of the soil affected the time period during which most of the larvae were recovered. The "great majority" of larvae was recovered from moist soil within 6 hr; however, a larger percentage of larvae was recovered from saturated soil between 6 and 24 hr. Most reports state that samples were baermannized for 24 or 48 hr. We found that putrefaction killed many of the larvae when grass and fecal samples were baermannized for more than about 24 hr. In our study most of the larvae were recovered by 6 hr, and relatively few were recovered between 6 and 24 hr.

Our work substantiated the fact that temperature influences the numbers of larvae recovered from the Baermann apparatus. Figure 2 illustrates that a temperature about that of most laboratories is the most efficient for recovering *H. contortus* larvae. Baermannization at lower temperatures did not give a significantly higher yield, and temperatures of 30 C and higher greatly decreased the numbers of larvae recovered. The optimum temperature evidently varies for different species of nematodes. Cort et al. (1922) found that the water used must be at least 10 F higher than the soil in order to obtain good recovery of *Necator americanus* larvae. Cort et al. (1926) reported that water temperatures between 35 and 45 C gave the best recovery for *Ancylostoma* and *Necator* larvae. Kauzal (1940) found that higher numbers of *H. contortus* and *Trichostrongylus* spp. larvae were recovered at temperatures between 5 and 22 C than at 37 C. Slightly higher numbers of *H. contortus* larvae were recovered at room temperatures of 22–26 C than in an incubator at 24–28 C. He found that recovery of *Trichostrongylus* spp. was about the same at these two temperature ranges. Adams (1965) found that the optimum temperature for recovery of soil nematodes was between 15 and 25 C. Higher or lower temperatures yielded fewer nematodes. His data indicated that temperature could affect the species of nematodes that were recovered in highest numbers.

We found that there was no advantage in using solutions other than water for baermannization. Rohrbacher (1957) reported that adding 0.5 ml of a nonionic detergent (Triton X-100) per liter of water increased the recovery of *Trichostrongylus axei* and *Ostertagia ostertagi* from Bermuda grass, orchard grass and crimson clover. We found that 0.2% solutions of the anionic detergents, Tergitol Wetting Agent No. 7, Tergitol Penetrant 4 and the nonionic detergent Tween-80, gave lower returns than tap water. Concentrations above 0.5% of these solutions were toxic to the larvae, and most were dead within 24 hr after having been placed in the solutions.

Placing the Baermann apparatus in the light or dark did not alter the numbers of larvae recovered. Kauzal (1940) attempted to attract *H. contortus* and *Trichostrongylus* spp. larvae to the base of funnels by blackening all but the lower stem of the funnels and by applying both light and heat to the neck of the funnels. Neither method gave better results than when the usual technic was used.

The results of the present study indicate that the size of the funnel is an important factor in determining the percentage of larvae recovered. According to Cort et al. (1922), Baermann (1917a) first used a small funnel and later (1917b) used a larger one which gave uniformly satisfactory results. Kauzal (1940) found that when 50 g of soil were placed in 6 inch funnels the mean recovery of *H. contortus* and *Trichostrongylus* spp. was 33.6%. When 100 g of soil were placed in 6- and 9-inch funnels, the mean recoveries were 17 and 43%, respectively. Mönnig (1930) reported that the best recovery of *Trichostrongylus* spp., *H. contortus* and *Oesophagostomum columbianum* larvae from fecal and soil samples was from 6 inch funnels and stated that if a large amount of material was to be examined it was better to use a number of funnels rather than a large funnel.

The effects of the amount and length of grass on baermannization have evidently not been reported in the literature. Our results indicate that when more than 50 g of longer grass was used there was a significant decrease in recovery. However, these data are misleading because the 50, 100 and 150 g samples were baermannized in 30 cm funnels, while the 5, 10 and 20 g samples were in 15 cm funnels. In experiment V 38% larvae were recovered from 30 cm funnels than from 15 cm funnels.

The influence of soil type on the results of baermannization has been studied by several authors. Our experiments confirm the previous reports that larger numbers of larvae are recovered from loose sandy soil than from clay soil. Baermann (1917b) reported that *Ancylostoma* larvae move through loose soil faster than through compact soil. Cort et al. (1922) obtained low recoveries of *Necator americanus* larvae from clay, but obtained increasingly better results with clay loam and sand, respectively. The closely packed particles of clay evidently hinder movement of the larvae between the spaces in the soil. Cort et al. (1922) found that mixing coarse gravel with clay loam greatly increased the percentage of larvae recovered. Stoll (1923) mixed known numbers of *N. americanus* larvae with humus, sand and loam, and clay, and recovered about 90, 80, and 30–50% of the larvae, respectively. Mönnig (1930) obtained "best results" with coarse soil and 6 inch funnels when he tested the efficiency of the Baermann technic with *Trichostrongylus* spp., *H. contortus* and *O. columbianum*.

Many technics other than or modifications of the Baermann apparatus have been described for recovery of nematodes from feces, grass and soil. Some authors have concluded that the Baermann technic should be used for qualitative rather than quantitative purposes. However, many of the other methods for determining the number of larvae present in soil, grass and fecal samples are time consuming and require elaborate equipment. We believe that the Baermann technic is a useful tool in determining the relative number of larvae present, if the investigator realizes its limitations.

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Monogenetic Trematodes from Costa Rica with the Proposal of *Anacanthocotyle* gen. n. (Gyrodactylidae: Isancistrinae)

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ABSTRACT: Four species of Gyrodactylidae are comparatively or originally described from Costa Rican fishes as follows: *Anacanthocotyle anacanthocotyle* sp. n. and *Gyrodactylus neotropicalis* sp. n. both from *Astyanax fasciatus* (Cuvier); and *G. costaricensis* sp. n. and *G. bullatarudis* Turnbull, 1956, both from *Poecilia sphenops* Valenciennes. *Anacanthocotyle* gen. n. is proposed. This genus differs from the related *Isancistrum* de Beauchamp, 1912, principally by possessing cephalic lobes which contain a spicule and portions of the head organs, 16 instead of 15 adult haptoral hooks, and a tapered peduncle on which a cup-shaped haptor occurs. *Anacanthocotyle* gen. n. lacks anchors and bars and possesses posteriorly confluent intestinal crura.

The description of *Cleidodiscus travassosi* and *C. chavarriai* from *Rhamdia rogersi* (Regan) was the first report of freshwater Monogenea from Costa Rica (Price, 1938). Recently, Price and Bussing (1967, 1968) described *C. costaricensis, C. strombicirrus* and *Palombitrema heteroancistrium* from *Astyanax fasciatus* (Cuvier) from Costa Rica. The present study includes four additional species from Costa Rican fishes and represents the first report of members of the Gyrodactylidae from the Neotropical Region.