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THE BAERMANN TECHNIQUE FOR ESTIMATING *PROTOSTRONGYLUS* INFECTION IN BIGHORN SHEEP: EFFECT OF LABORATORY PROCEDURES

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ABSTRACT: The modified Baermann funnel technique was evaluated to determine the effects of time of baermannization, fecal preparation, type and size of funnel, and type of filter on the number of first stage larvae of *Protostrongylus* spp. recovered from feces of Rocky Mountain bighorn sheep (*Ovis canadensis*). More larvae were recovered when fecal pellets were baermannized for 24 hr compared to 8 hr, and when feces were crushed than when left intact. Use of small funnels resulted in the recovery of more larvae per gram of feces than larger funnels, and glass funnels more than plastic ones. There was no difference in recovery of larvae between cheesecloth filters and cellulose filters.

The Baermann funnel was first described in 1917 as a method to extract hookworm larvae from soil (Baermann, 1917). A modified Baermann funnel was developed in 1922 (Cort et al., 1922) and is used widely to recover nematodes from soil, grass, and feces (Todd et al., 1970). The Baermann apparatus has been further adapted to increase efficiency of recovering specific nematodes. These adaptations have been evaluated for extraction of larval stages of *Haemonchus contortus* (Dinaburg, 1942; Todd et al., 1970), hookworms (Cort et al., 1922), and *Protostrongylus* spp. (Pillmore, 1958, 1959, 1961).

Several variations of the modified Baermann apparatus have been routinely used by the Colorado Division of Wildlife for determining infections of *Protostrongylus* spp. in Rocky Mountain bighorn sheep (*Ovis canadensis*). Because minor variations in techniques of counting may affect results (Levine et al., 1960), reliable comparison of data obtained by different variations of the Baermann technique is difficult. We examined the effects of laboratory protocol on isolation of first stage larvae of *Protostrongylus* spp. from feces of bighorn sheep.

METHODS AND MATERIALS

The effects of laboratory procedures on recovery of larvae were assessed in two experiments. In Experiment 1 we used a three-way factorial design with nine replications per cell to examine effects of funnel diameter (15 vs. 6.3 cm), sample preparation (crushed vs. whole feces) and baermannization time (8 vs. 24 hr). Experiment 2 was a two-way factorial with 12 replications of two levels of funnel type (20 cm glass vs. 15 cm plastic) and two levels of filter type (cheesecloth vs. cellulose tissue). Significance of treatment effects and interactions was examined with analysis of variance.

In both experiments, fecal pellets containing first stage larvae of *Protostrongylus* spp. were collected from a captive, castrated bighorn sheep between 22 February and 19 March 1980. Collections were made from a single bighorn sheep to minimize individual variation in level of infection. All fecal samples were collected immediately after defecation and were obtained at random throughout daylight hours. Fresh feces were composited in a paper bag, thoroughly mixed and stored in a freezer at −20 C, for a maximum of 6 wk. Subsamples of fecal pellets were removed from the freezer and thoroughly mixed at the time of each replication.

We used a standard Baermann apparatus consisting of a funnel (glass or plastic, depending on treatment) with a 40-mesh brass wire screen placed approximately 3 cm below the top of the funnel. A rubber tube was connected to the funnel bottom and a clamp attached to the tube approximately 10 cm below the funnel. The funnel was filled with warm tap water (20–28 C), and a filter placed over the wire screen. In Experiment 1, cellulose tissue ("Kimwipes," Kimberly-Clark Corp., Neenah, Wisconsin 54956, USA) was used for filters in all funnels. In Experiment 2, half of the funnels received cellulose filters and the other half, two layers of cheesecloth. Baermannization time was varied (8 vs. 24 hr) in Experiment 1 and was held constant (24 hr) in Experiment 2.

Procedures for preparing feces and counting larvae were identical in the two experiments. Frozen feces were left at room temperature for 20 to 30 min to allow for frost to evaporate. Five g (wet wt) feces, weighed to the nearest 0.01 g, were added to all funnels, except the 6.3 cm funnels which received 1.00 g. Feces were spread evenly on the filter and covered with the edges of the filter to prevent floating. After baermannization, slightly less than 100 ml of fluid was drawn off from the bottom of the funnel,

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TABLE 1. Effect of funnel size, sample preparation, and baermannization time on counts of *Prostrongylus* larvae in bighorn sheep feces.

Laboratory procedure	Larvae/g of wet weight feces		
	$\bar{x}$[a]	95% CI[b]	P[c]
Funnel size[d]			0.05
15 cm	130	116–144	
6.3 cm	149	135–163	
Sample preparation			0.0001
Whole	58	44–72	
Crushed	221	207–235	
Baermannization time			0.0001
8 hr	89	75–103	
24 hr	190	176–204	

[a] One-way means averaged over other factors.
[b] 95% confidence interval calculated as $\pm t_{0.95,2} \times$ pooled error of one-way means.
[c] Significance level of main effects. Two- and three-way interactions were not significant ($P > 0.25$).
[d] Both funnels were made of glass.

and tap water added to constitute a sample of 100 ml. This sample was thoroughly stirred and two 5 ml aliquots withdrawn with a pipette. This subsample was placed in a petri dish (internal dimensions 95 mm × 95 mm) with a grid etched on its bottom to facilitate counting. Larvae were identified by comparing them with descriptions and illustrations given by Pillmore (1955), and counted under a dissecting microscope. For each 100 ml sample, two separate counts were obtained and a mean calculated and converted to larvae per g of wet fecal material. If the two counts differed greatly, a third count of a 10 ml subsample was obtained and a mean calculated for three subsamples.

RESULTS

Experiment 1: Funnel size exerted a small but significant ($P = 0.05$) effect on recovery of larvae by the Baermann technique; counts from 6.3 cm glass funnels were 15% higher than counts from 15 cm glass funnels (Table 1). Influences of sample preparation and baermannization time were highly significant ($P < 0.0001$). Crushed feces yielded counts almost four times higher than counts from whole feces. Increasing baermannization time from 8 to 24 hr doubled the recovery of larvae. Magnitude of differences in treatment means was constant over all treatments (2-way interactions $P > 0.50$, 3-way interaction $P = 0.25$).

Experiment 2: Filter type had no effect on number of larvae recovered ($P = 0.99$, Table 2). However, counts of larvae were markedly influenced by funnel type ($P < 0.001$); baermannization in 20 cm glass funnels resulted in

TABLE 2. Effect of filter and funnel type on counts of *Protostrongylus* larvae in bighorn sheep feces.

Laboratory procedure	Larvae/g wet weight feces		
	$\bar{x}$[a]	95% CI[b]	P[c]
Filter type			0.99
Cellulose tissue	249	229–268	
Cheesecloth	251	231–271	
Funnel type			0.0001
Plastic	212	193–232	
Glass	284	266–304	

[a] One-way means averaged over other factor.
[b] 95% confidence interval calculated as $\pm t_{0.95,2} \times$ pooled error of one-way means.
[c] Significance level of main effects. Two-way interaction was not significant ($P = 0.99$).

counts 34% higher than counts from samples baermannized in 15 cm plastic funnels. Effects of funnel type were independent of filter type (2-way interaction $P = 0.99$).

DISCUSSION

Because fecal samples were collected during a 4 wk period, they were frozen to minimize migration of larvae from feces and variation in sample drymatter resulting from the length of time samples were stored. Although freezing may have affected recovery of larvae by killing individuals, we assume this effect was constant across treatments and did not alter our comparisons.

Increased extraction of first stage larvae of *Protostrongylus* spp. with 24 hr baermannizations compared to 8 hr baermannizations was similar to results of other studies involving *Protostrongylus* spp. (Pillmore, 1958) and hookworms (Cort et al., 1922). Most larvae of *H. contortus* were recovered by 6 hr (Dinaburg, 1942; Todd et al., 1970), but an appreciable number were recovered later (Dinaburg, 1942).

In contrast to these similarities, our observation that greater recovery of larvae was obtained using crushed pellets is divergent with the findings of Todd et al. (1970) who reported similar counts from crushed and whole pellets. This difference may be explained by variations in the methods of crushing samples; Todd's crushed samples were so turbid that samples had to be centrifuged and washed before larvae could be easily counted (Todd et al., 1970). We found that slightly crushing feces, so that pellets were broken but still intact, produced a cleaner, more easily counted sample.

We observed no effect of filter type on recovery of larvae. Todd et al. 1970) using infective larvae of *H. contortus*, found a single layer of cheesecloth was a more efficient filter than cellulose tissue. Cort et al. (1926) used one and two layers of cloth in funnels and obtained slightly better results using one layer of cloth.

Funnels affected recovery of larvae in two ways. Similar to other reports (Cort et al., 1922; Todd et al., 1970), recovery was inversely related to funnel diameter. However, large funnels were only slightly less efficient in recovery than smaller ones, so small differences in funnel size should not cause excessive variation in larval counts. Further, despite the smaller fecal samples in 6.3 cm diameter funnels, relative variability in recovery between the two procedures was similar (coefficient of variation for 6.3 cm = 7.3%, for 15 cm = 7.7%). Unlike funnel size, funnel material had pronounced effects on larval counts. Since plastic is softer, more porous, and more easily abraded than glass, it may offer a "rougher," more complex surface to which larvae adhere during baermannization. Although funnel type was slightly confounded with funnel size (glass funnels were 20 cm, plastic 15 cm), the observation that *larger* funnels gave greater recovery than smaller plastic funnels, but less counts than smaller glass ones, confirms the effect of funnel material.

We conclude that reliable comparison of Baermann counts of larvae of *Protostrongylus* spp. in bighorn sheep feces depends on properly standardized laboratory procedures. In particular, funnel type, baermannization time, and sample preparation must be uniform to assure Baermann counts are comparable; we recommend that crushed fecal samples be baermannized for 24 hr in 15 cm glass funnels.

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