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***Parelaphostrongylus tenuis* in Maine Moose and the Possible Influence of Faulty Baermann Procedures**

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ABSTRACT: Efficacy of cleaning Baermann apparatus was evaluated to determine if larvae are retained on glassware after evaluating white-tailed deer (*Odocoileus virginianus*) fecal samples containing *Parelaphostrongylus tenuis*. Residual *P. tenuis* larvae were recovered from 7 (11.7%) of 60 Baermann apparatus cleaned with soap and tap water. Of 295 moose (*Alces alces*) fecal samples collected in central and northern Maine, only one contained protostrongylid larvae. Our data do not support the hypothesis that recent increases in Maine's moose population can be attributed to moose becoming a suitable host to *P. tenuis*.

Key words: *Parelaphostrongylus tenuis*, meningeal worm, *Alces alces*, moose, Baermann technique, Maine.

White-tailed deer (*Odocoileus virginianus*) are considered to be the only normal definitive host for the meningeal worm, *Parelaphostrongylus tenuis* (Anderson and Prestwood, 1981). *Parelaphostrongylus tenuis* generally is recognized as a pathogenic agent causing debilitating neurologic disease in moose (*Alces alces*) on range sympatric with infected white-tailed deer (Anderson and Prestwood, 1981; Lankester, 1987).

The Baermann apparatus is used routinely for detecting the presence of protostrongylid infections in cervids. Although laboratory protocol for the Baermann technique has been evaluated (Todd et al., 1970; Samuel and Gray, 1982; Beane and Hobbs, 1983), the importance of cleaning the apparatus between tests has not been addressed. Insufficiently cleaned Baermann apparatus may retain larvae when moose fecal samples are run consecutively with deer fecal samples infected with *Parelaphostrongylus* spp. *Parelaphostrongylus tenuis* larvae, first reported in moose feces collected on deer-free Isle Royale, Michigan (USA) (Karns and Jordan, 1969),

were believed to be the result of contaminated glassware (Lankester 1987). Other reports of unexpectedly high levels of *P. tenuis* larvae (10 to 12%) in moose feces from Maine (Clark and Bowyer, 1986) and Nova Scotia (Thomas and Dodds, 1988) also prompted us to question whether contaminated Baermann funnels could give false positive results.

Our objectives were to 1) determine if glassware contamination can occur from inadequate cleaning and 2) determine the presence or absence of larval *P. tenuis* in Maine moose.

The efficacy of cleaning Baermann apparatus was assessed by evaluating 60 deer fecal samples, infected with *P. tenuis*, for 24 hr with a Baermann apparatus. After the evaluations, the fecal sample and cheesecloth were discarded. Thirty of the apparatus then were cleaned with a brush and soap (SPF Liquid Hand Soap, Chute Chemical Co., Bangor, Maine) and rinsed with warm (20 to 28 C) tap water. Thirty apparatus were rinsed only with warm tap water. Immediately after cleaning, all empty funnels were re-filled with only warm tap water. After 24 hr, approximately 15 ml of fluid were drawn from the bottom of the funnel into a gridded Petri dish and larvae were counted under a dissecting microscope.

Our Baermann apparatus consisted of 7.5 and 10.5 cm diameter glass funnels with an 8 cm plastic tube attached to the funnel bottom. A clamp was attached to the tube approximately 6 cm below the funnel. Deer and moose fecal samples (50 to 100 g) were wrapped in a double layer of cheesecloth, placed in the funnel, and hydrated with warm tap water.

All of the 60 deer fecal samples con-

tained 2 to 199 *P. tenuis* larvae/g feces. Small numbers of protostrongylid larvae ($n = 1$ to 3) settled out of 4 (13.3%) of 30 funnels subsequently cleaned with soap and water and 3 (10.0%) of 30 funnels rinsed with tap water only.

Thus, Baermann apparati sometimes retain protostrongylid larvae after cleaning with soap and water. We suspect larvae cling to the glassware or the rubber tubing attached to the funnels. Plastic funnels might retain even more larvae because of their porous, more easily abraded surface (Beane and Hobbs, 1983). We found that *P. tenuis* larvae remained viable ≤ 22 days on uncleaned Baermann glassware after drying at 22 to 28 C (M. McCollough and K. Pollard, unpubl.). In light of these findings we recommend that Baermann apparati be autoclaved prior to each use, especially when concurrently evaluating the occurrence of protostrongylid infections in several species of cervids.

From December to April, 1987 to 88 and 1988 to 89, 295 moose fecal samples were collected in 27 townships throughout Maine's moose range (Fig. 1). Fifty-eight fecal samples were collected in Baxter State Park, including the area sampled by Clark and Bowyer (1986). Feces were collected opportunistically over a 10 to 100 km² area within each site to avoid multiple sampling of individual moose. Most fecal samples were collected from fresh snow and stored frozen for ≤ 2 mo.

Larvae were extracted using a modified Baermann technique (Todd et al., 1970) for 24 hr. Baermann apparati were washed and autoclaved prior to each use, and new petri dishes were used for each test. Larval identification was verified by H. Gibbs (Univ. of Maine, Orono, Maine) and M. Lankester (Lakehead Univ., Thunder Bay, Ontario, Canada).

We found first-stage protostrongylid larvae in only one of 295 moose fecal samples. The sample came from central Maine (Sebois Plantation, 45°24'N, 68°45'W). Clark and Bowyer (1986) reported that 9.6% of moose fecal groups ($n = 594$) from

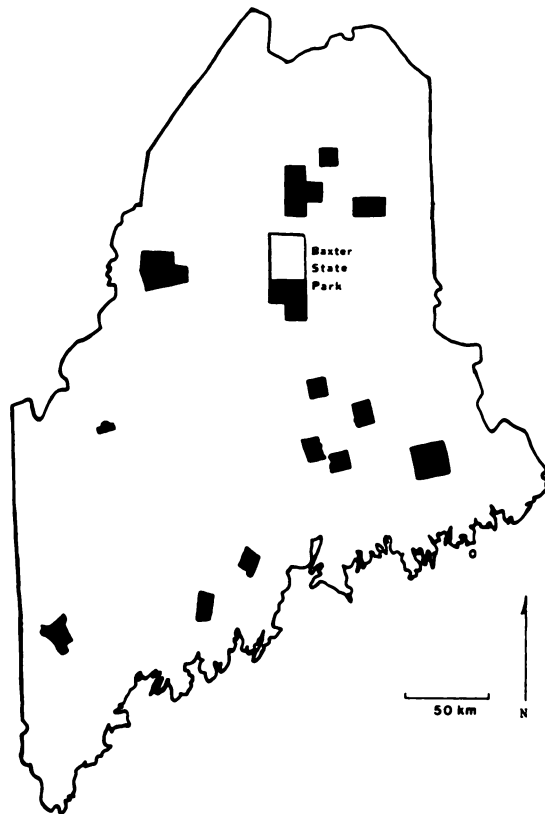


FIGURE 1. Location of moose fecal sampling sites in Maine.

Baxter State Park in north-central Maine were infected. None of our 58 samples collected in the same area contained protostrongylid larvae.

Our data do not support Clark and Bowyer's (1986) hypothesis that moose and *P. tenuis* in Maine are coevolving and causing a reduction in the debilitating effect of *P. tenuis* on moose. Similarly, in neighboring New Brunswick, Upshall et al. (1987) did not find protostrongylid larvae in 61 moose fecal samples examined. However, our observation of a single moose shedding larvae does complement evidence that *P. tenuis* can complete its life cycle in moose. Bogaczyk (1990) and M. Lankester (pers. comm.) documented larvae in feces of moose with signs of parelaphostrongylosis and recovered adult *P. tenuis* from the meninges. We conclude that a single moose found shedding pro-

tostrongylid larvae in this study probably was *P. tenuis* in an aberrant host.

Some reports of protostrongylid larvae in moose feces may be the result of Baermann contamination. It is difficult to interpret the results of any Baermann analysis unless the authors specify their methods in sufficient detail.

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