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The Potential for False-positive Diagnosis of Protostrongyliasis by Extraction of Larvae from Feces

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ABSTRACT: The potential of protostrongylid first-stage larvae (L_1) to survive passage through the alimentary canal of non-infected mammals was investigated. *Parelaphostrongylus tenuis* L_1 were collected from feces of an experimentally infected white-tailed deer (*Odocoileus virginianus*). We utilized two red deer (*Cervus elaphus*) and four laboratory rats (*Rattus norvegicus*) which were each fed the L_1 of *P. tenuis*. Larvae were recovered, intact and alive, from the fecal samples of all six animals. Larvae of *P. tenuis*, and probably of other related species, can survive passage through the alimentary canal of uninfected mammals and they can be collected from feces using the Baermann technique and other related larval extraction methods. Rain water was found to be successful in the dispersal of *P. tenuis* L_1 from the feces of infected animals. These findings raise the possibility of ingestion of L_1 and their subsequent passage, by uninfected animals. This potential for false-positive diagnosis of infection in live animals necessitates accurate interpretation of a host's infection-status. Such findings reinforce the need for a reliable method of diagnosing infections in live animals.

Key words: Baermann method, dispersal, false-positive diagnosis, feces, first-stage larvae (L_1), *Parelaphostrongylus tenuis*, rain.

Parelaphostrongylus tenuis is a protostrongylid nematode commonly found infecting white-tailed deer (*Odocoileus virginianus*) of eastern North America (Anderson and Prestwood, 1981). First-stage larvae (L_1) are passed with the fecal pellets from infected deer. These L_1 are required to infect molluscs and undergo development to the third larval-stage (L_3) in order to become infective to deer, and ultimately for the life-cycle to continue (Anderson and Prestwood, 1981).

The Baermann funnel method has been widely used as a successful means of collecting nematodes from soil, vegetation, and feces (Todd et al., 1970). More precisely, it has often been used to collect first-stage larvae (L_1) of protostrongylid nematodes from feces of infected cervids such as deer (*Odocoileus* spp.), moose (*Alces alces*), elk (*Cervus elaphus*) and caribou (*Rangifer tarandus*) (Forrester and Lankester, 1997). To date it has been assumed that the L_1 collected from a cervid's feces are the result of an existing infection in that cervid. The possibility of a cervid passing live, through its alimentary canal, any L_1 which may be ingested from the environment has not previously been investigated. In the present study, this assumption was examined. Under natural conditions the L_1 are thought to leave the mucous coat of fecal pellets through dispersal during heavy rains or snow melt (Lankester and Anderson, 1968). Thus, we briefly examined the effect of rainfall on L_1 dispersal prior to examining L_1 passage through the alimentary canal.

Fecal pellets were collected from a white-tailed deer that previously had been experimentally infected with *P. tenuis*. The fecal pellets (about 5 g) were placed on nylon window screening (Forrester and Lankester, 1997) which was, in turn, placed in a clean (Whitlaw and Lankester, 1995) 15 cm glass funnel. This set-up, so designed that rain water could pass over the feces and into a collection container, was placed outside during a rain storm in April, 1997. The rain water was collected after 24 hr and examined for presence of the L_1. In order to enumerate the remaining larvae, the feces and window screening were removed from the funnel and placed into a water-filled beaker (Forrester and Lankester, 1997) for a further 24 hr, after which time the water was examined for the presence of L_1. The rain water contained 460 L_1. A further 1,869 L_1 were recovered from the beaker. A similar total of L_1 (2,610) were extracted from an additional

5 g of feces from the same pellet group using only the method of Forrester and Lankester (1997). Thus, approximately 20% of the larvae were removed from the feces by rainfall over a 24 hr period.

Larvae of *P. tenuis* were collected from the feces of an experimentally infected white-tailed deer using a modification of the Baermann technique of Samuel and Gray (1982) for collecting protostrongylid larvae from feces. Of these L_1, 3,000 were fed to each of two laboratory-born and raised adult white rats (*Rattus norvegicus*) and each of two red deer (*Cervus elaphus*) that were maintained in captivity for 2.5 yr. Dorsal-spined nematode larvae were not recovered from any of the biweekly fecal samples from either red deer prior to this experiment. A replicate L_1 feeding experiment using the same exposure in the same two red deer also was performed. The entire daily fecal output of each of the four animals was collected from the floor of their individual pens. Rat feces was collected for three days beginning at one day pre-exposure to the L_1. Red deer feces was collected for 9 days beginning at 2 days pre-exposure to the L_1. In the replicate feeding of red deer, feces was only collected for 4 days beginning at 2 days pre-exposure to the L_1. Control animals were not included as the pre-exposure and later post-exposure fecal samples served as controls for each individual animal. Daily fecal samples (6–12 g for rats and 1,200–1,800 g for red deer) were examined for the presence of L_1 by the conventional Baermann method with a maximum of 25 g of feces per funnel. The procedure was repeated using two additional laboratory rats, but this time each rat was fed 2,000 L_1 of *P. tenuis* and larvae were collected from their feces using the method of Forrester and Lankester (1997).

Active L_1, consistent in size and morphology with those of *P. tenuis*, were recovered from the feces of both red deer in each replicate. One hundred sixty two and 113 L_1 were recovered from the feces of one deer in the two replicates, respectively, whereas 32 and 39 L_1 were recovered from the two replicates of the other red deer, respectively. The larvae were recovered by 24 hr post-feeding, the one exception being that two of the 162 L_1 recovered in total from one red deer were collected from the 24 to 48 hr fecal samples. More of the initial L_1 exposure may have been recovered had we used the method of Forrester and Lankester (1997) instead of the conventional Baermann method. Extraction of L_1 from rat feces using the conventional Baermann technique resulted in recovery of 72 and 39 L_1 from each rat, respectively, whereas recovery from feces of the additional two rats using the method of Forrester and Lankester (1997) was 340 and 116 L_1, respectively. All of the L_1 were recovered from rats by 24 hr post-feeding. Many of the L_1 recovered from the rat feces appeared dead although some were still alive. The red deer were subsequently euthanized and a necropsy performed as part of a coincidental study. No evidence of protostrongylid infection was found in either animal.

Larvae of *P. tenuis*, and probably of other related species, can survive passage through the alimentary canal of uninfected mammals and they can be collected from feces using the Baermann method, and other related larval extraction methods. Since the majority of larvae were recovered from the red deer by 24 hr post-feeding, this finding might mean that clearance of L_1 from the alimentary canal takes only about 24 hr regardless of whether L_1 originate from within the cervid or they are the result of "free passage" from ingestion of L_1 originating from a different infected animal.

Our findings support the fact that presence or absence of the L_1, by extraction from cervid feces, is not a reliable diagnosis of whether an animal is infected with a protostrongylid nematode. Presently, in the absence of an accurate diagnostic method, the true status of infection of any cervid can be determined only by collection of adult worms through necropsy

(Prestwood et al., 1974; Pybus and Samuel, 1981; Samuel, 1987). Our findings support Lankester and Anderson's (1968) suggestion that rain water can successfully disperse L_1 from fecal pellets and also show that if such living dispersed larvae were ever ingested by another cervid, they could pass unscathed through its alimentary canal and thereby provide a false-positive diagnosis of infection if recovered by larval extraction from feces.

Although it is difficult to predict the frequency of ingestion of L_1 from the environment, our findings provide another reason that diagnostic conclusions cannot be drawn based on single fecal samples or in cases where larval recovery is low. The implications of our findings in surveys of wild cervid populations are probably limited. In farmed animals, a false-positive diagnosis could be attributed to infections in surrounding populations of wild cervids or in pen-mates. The potential for ingestion and free passage of L_1 should be considered in experimental studies where different species or treatment groups are housed together or in close proximity. An example of a potential source of contamination comes from our experience with captive cervids which has shown that animals defecate in the water supply on a fairly regular basis. These findings reinforce the need for a reliable antemortem diagnostic method.

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