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Meningeal Worm is a Long-lived Parasitic Nematode in White-tailed Deer

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ABSTRACT: A natural infection of the meningeal worm, *Parelaphostrongylus tenuis,* persisted for at least 3.7 yr in a white-tailed deer (*Odocoileus virginianus*). The deer was 5–7 yr old and was shedding dorsal-spined nematode larvae at the time of quarantine. Larvae were extracted from all fecal samples collected up to 730 days post-quarantine (dpq) and thereafter only at 862 dpq and at necropsy (1,350 dpq). Live adults of *P. tenuis,* one male and one female, were recovered from the cranium at necropsy. *Parelaphostrongylus tenuis* infections are long lived and latent periods may be extended. Our findings reaffirm the need for reliable antemortem diagnosis to identify non-patent *P. tenuis* infections to prevent inadvertent introduction of infected animals to non-endemic areas.

Key words: Baermann method, corticosteroids, diagnosis, larval extraction, life span, meningeal worm, *Parelaphostrongylus tenuis,* patent periods, white-tailed deer.

Parelaphostrongylus tenuis is a common nematode parasite of white-tailed deer (*Odocoileus virginianus*) in eastern North America (Anderson and Prestwood, 1981). Tentative diagnosis of infection can be made through extraction of dorsal-spined first-stage larvae (L_1) from feces. However, related nematode parasites of white-tailed deer have L_1 with similar morphology (Anderson, 1992). Definitive diagnosis requires recovery of adult nematodes at necropsy (Gray et al., 1985). Related elaphostrongyline nematodes are long-lived (Watson, 1984). Although definitive information is lacking, *P. tenuis* may persist for the life of its host (Peterson and Lankester, 1991; Slomke et al., 1995).

White-tailed deer infected with *P. tenuis* typically pass L_1 in feces. There is periodicity in the intensity of larval shedding based on age of the animal and season (Peterson and Lankester, 1991; Slomke et al., 1995). A similar seasonality of larval shedding was documented for related elaphostrongyline nematodes (Wissler and Halvorsen, 1976; Samuel et al., 1985) and this shedding may be associated with seasonal physiologic stress (Gaudernack et al., 1984).

An adult male white-tailed deer was obtained from a wildlife park in Saint John, New Brunswick, Canada in 1992. Upon acquisition, this animal had a patent protostrongylid infection revealed by dorsal-spined first-stage nematode larvae (L_1) in feces. The deer was quarantined in an indoor facility with a cement floor to ensure no contact with terrestrial gastropods and was maintained for 3.7 yr. Natural photoperiod was maintained using artificial light, and the temperature varied with that of the outside ambient temperature. Fecal samples were collected either directly from the rectum or immediately after the pellets were dropped on the floor of the pen. Feces were collected, on average, every 2–3 wk throughout the study. Approximately 15 g of fecal pellets were placed in a Baermann apparatus (Samuel and Gray, 1982) for 18–24 hr at room temperature. Fluid (15 ml) from each sample was collected, concentrated by centrifugation, and examined (25×) for the presence of L_1.

The deer received an intramuscular injection of dexamethasone (0.1 mg/kg; Azium, Schering Canada Inc., Point-Claire, Quebec, Canada) every 3 days for a total of five injections (UNB Animal Care

Protocol # 96035) beginning 1,308 days-post quarantine (dpq). Fecal samples were collected on the day of each injection and biweekly until euthanasia. Dexamethasone injections were discontinued after the fifth injection due to an *Actinomyces* sp. infection of the right front fetlock. Antibiotics (10 cc; Pen-Di-Strep, Rogar/STB, London, Ontario, Canada) were given daily for 12 days post-corticosteroid treatment. The deer was euthanized 18 days later at 1,350 dpq. The white-tailed deer was anesthetized by intramuscular injection of 3 mg/kg xylazine (Rompun, Miles Canada Inc., Etobicoke, Ontario) and euthanized by intravenous injection of 20 ml of euthanasia solution (T-61, Hoechst Canada Inc., Laval, Quebec, Canada).

Brain and spinal cord were removed and examined grossly for nematodes. The dura mater was examined in situ and was then removed. The sagittal, transverse, and cavernous cranial venous sinuses were dissected carefully in phosphate buffered saline (PBS; 0.01 M sodium phosphate, 0.145 M NaCl, pH 7.2) while viewing with a dissecting microscope (6–12×). The central nervous system (CNS) and meninges were soaked in PBS at room temperature for 24 hr. The resultant fluid was washed through an ethanol-sterilized 38-μm sieve (English et al., 1985) and the retentate examined at 25× for the presence of nematodes or nematode larvae. The entire CNS was washed through a 250-μm sieve until only the meninges remained.

Lungs were examined for *Varestrongylus alpenae* by palpation for nodules and laborious dissection to open all bronchioles (Gray et al., 1985). The lungs were then soaked in tap water for 24 hr at room temperature and the resultant fluid washed through an ethanol-sterilized 38-μm sieve (English et al., 1985) and the retentate examined as above. The entire 84.5 g of colonic feces were processed using the Baermann method with a maximum of 15 g per funnel. The appendicular musculature was dissected carefully and individual muscles were examined grossly for nematodes. The axial musculature of the spinal column (epaxial and sub-vertebral musculature) was removed from the skeleton and frozen. The muscles were examined for *P. andersoni* by viewing thin sections (ca. 5 mm) with a light magnifier and dissecting microscope (Prestwood et al., 1974). The gastrointestinal tract was examined for helminths using standard parasitologic techniques. An incisor tooth (I1) was extracted and the age determined by cementum analysis (Matson's Lab, Milltown, Montana, USA).

Larvae were extracted and counted from all fecal samples collected 1–304 dpq (0.1–33.8 L_1/g feces). Larvae were extracted from all fecal samples 310–730 dpq but were not counted. Larvae were not detected again until 862 dpq (2.0 L_1/g feces). Larvae were not extracted from any other fecal samples either prior to or during corticosteroid treatment, nor prior to euthanasia. A single L_1 was recovered from the colonic feces collected at necropsy. The washings of the brain and meninges contained 14 live L_1. Larvae were not recovered from the spinal cord. Nodules and adult nematodes were not detected in the lungs. Washings of the lung contained a single live L_1, but others may have been missed due to postmortem contamination by rumen contents.

An adult male and female *P. tenuis,* both alive, were recovered from the subdural space above the right cerebral hemisphere of the brain. Nematodes were not found in the musculature. The deer's age was estimated to be 9–11 yr old indicating that the deer was 5–7 yr old when first quarantined in the cement-floor pen facility.

This study represents the first definitive report on the life span of a *P. tenuis* infection. This white-tailed deer had a patent natural infection at the time of acquisition. Life span was determined based on the last possible day that this animal could have been exposed to infective larvae (L_3) of *P. tenuis,* which was the day on which it entered the indoor cement-floor pen facility. Our results indicate that *P. tenuis*

can survive 3.7 yr in white-tailed deer and probably much longer. Unfortunately, the entire history of this animal is unknown. Survey data from New Brunswick, Canada, indicates that 86% of wild white-tailed deer are infected with *P. tenuis* in their first year of life (Duffy, 2000). The time of acquisition of the infection in our deer is unknown, but it may have been 4.5–6.5 yr prior to quarantine. This would not be surprising because related nematodes are reportedly long-lived. Samuel (1987) described an anecdotal report of a *P. odocoilei* infection in a captive mule deer (*O. hemionus hemionus*) persisting for 9 yr. Based on the static number of adult worms in adult white-tailed deer of increasing age classes, Slomke et al. (1995) proposed that adult *P. tenuis* are long-lived.

Recovery of live L_1 at necropsy indicated that the adult worms were reproducing up to 3.7 yr, albeit at a reduced rate compared to earlier in the infection. The L_1 were undoubtedly those of *P. tenuis* because no other protostrongylid nematodes were recovered at necropsy. In addition, larvae collected from this animal during its first 2 yr in captivity were used to infect terrestrial gastropods, and in turn, the recovered L_3 were used experimentally to infect white-tailed deer. These deer were subsequently euthanized and *P. tenuis* was the only protostrongylid nematode recovered at necropsy (unpubl. data).

Seasonal factors (i.e., daylight and temperature) did not appear to influence the latent periods in our study because larvae were not recovered from feces for >1 yr. Larvae may have been passed during this latent period, but at a level too low for detection using the Baermann method (Forrester and Lankester, 1997). It is also possible that this infection could have produced detectable larvae on days that the feces were not examined. In addition, if the deer had been maintained for a longer time more larvae might have been shed.

Conclusions about the effects of corticosteroids on non-patent *P. tenuis* infections cannot be made, except that in this case larval shedding was only detected at necropsy. The corticosteroid treatment may have played a part in reinitiating larval shedding by adult *P. tenuis*, based on recovery of L_1 from the CNS, lungs, and feces at necropsy. It is more likely, however, that female worms have a decreased reproductive viability with increasing age and that immune mechanisms of the host prevented active shedding of L_1 with the fecal pellets. Slomke et al. (1995) suggested that the lower number of larvae in feces from older white-tailed deer may be the result of decreased fecundity of aged worms or immune mechanisms directed towards eggs and L_1. Alternatively, this decreased reproductive viability may be due to a direct effect on adult worms, whereby adult female *P. tenuis* are unable to enter blood vessels and release fertilized eggs. Larvae arising from eggs deposited within the CNS likely have decreased survival based on the limited survival time of *P. odocoilei* L_1 at 37 C (Shostak and Samuel, 1984).

Our findings have important implications for diagnosis of *P. tenuis* infections. A polymerase chain reaction (PCR)-based diagnostic assay has recently been proposed for diagnosis of *P. tenuis* and related species (Gajadhar et al., 2000). This PCR assay and conventional larval extraction methods rely upon the presence of L_1 in host feces. The periodicity of larval shedding (Peterson and Lankester, 1991; Slomke et al., 1995) and potential for extended latent periods in *P. tenuis* infections, as we describe, limit the usefulness of such assays as diagnostic tools. Attempts to develop a reliable antemortem serologic assay for *P. tenuis* infections in cervids (Dew et al., 1992; Duffy et al., 1993; Neumann et al., 1994; Bienek et al., 1998; Ogunremi et al., 1999a, b) have also been described. In each instance, sera were only obtained from deer infected ≤0.5 yr previously. Serologic tests need to be validated during both patent and latent infections and, as evidenced by this study, in animals with old infections.

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