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Parelaphostrongylus tenuis–Associated Meningoencephalitis in a Sika Deer (*Cervus nippon*)

Richard W. Gerhold,[1,4,5] M. Kevin Keel,[1] Kim Arnold,[2] Doug Hotton,[3] and Robert B. Beckstead[4]
[1] Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA; [2] Maryland Department of Agriculture, Salisbury, Maryland 21801, USA; [3] Maryland Department of Natural Resources, Salisbury, Maryland 21801, USA; [4] Department of Poultry Sciences, The University of Georgia, Athens, Georgia 30602, USA; [5] Corresponding author (email: rgerhold@uga.edu)

ABSTRACT: An adult, female, free-ranging, sika deer (*Cervus nippon yakushimae*) from Wicomico County, Maryland, USA, was found circling and having no fear of humans. The animal was euthanized and submitted for a postmortem exam. There were no gross lesions and the deer was negative for rabies. Microscopic examination revealed lymphoplasmacytic, neutrophilic, and eosinophilic meningoencephalitis with intralesional adult nematodes, larvae, and eggs consistent with nematodes in the family Protostrongylidae. *Parelaphostrongylus tenuis* was identified by polymerase chain reaction and DNA sequencing. To our knowledge, this is the first report of *P. tenuis*–associated encephalitis in a sika deer.

Key words: *Cervus nippon yakushimae*, *Parelaphostrongylus tenuis*, sika deer.

Parelaphostrongylus tenuis–associated disease has been reported from numerous domestic and wild mammals in the families Bovidae, Camelidae, and Cervidae (Anderson, 2000; Lankester, 2001) and was suspected as the cause of disease in a horse (*Equus caballus*; Tanabe et al., 2007). White-tailed deer (*Odocoileus virginianus*) are the definitive host of *P. tenuis* and terrestrial slugs and snails are required intermediate hosts. No reports of parelaphostrongylosis in sika deer (*Cervus nippon*) have been published. We report a case of acute meningoencephalitis due to *P. tenuis* infection in a free-ranging sika deer.

On 14 February 2007, a female, free-ranging sika deer in Wicomico County (38°22′N, 75°36′W), Maryland, USA, was reported circling in a parking lot and having no fear of humans. The deer was euthanized by personnel of the Maryland Department of Natural Resources and submitted to the Maryland Department of Agriculture's Salisbury Laboratory, Salisbury, Maryland for necropsy. The entire head and sections of spleen, liver, and lung were refrigerated and submitted to the Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia, USA, for diagnostic examination.

The entire cerebrum and brain stem and portions of the cerebellum, lung, liver, and spleen were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin (H&E) for light microscopy. Portions of the lung, liver, and spleen were collected and frozen at −20 C. A portion of cerebellum was submitted to the Athens Diagnostic Laboratory for rabies virus testing by fluorescent antibody (FA) assay.

Three 10-μm sections of formalin-fixed, paraffin-embedded brain tissue, containing protostrongylid larvae, were processed for DNA extraction using QIAamp® DNA Mini kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. Primers used for polymerase chain reaction (PCR) were 5′-CCGTCGAATACATGTCATCC-3′ and 5′-TCGTCAAGACGATGATTCCC-3′ (Gajadhar et al., 2000). These primers amplify a 228–base-pair (bp) product covering a portion of the second internal transcribed spacer (ITS-2) region of the ribosomal RNA (rRNA) gene in *Parelaphostrongylus* spp. The reaction mixture included 10 mM Tris-HCl

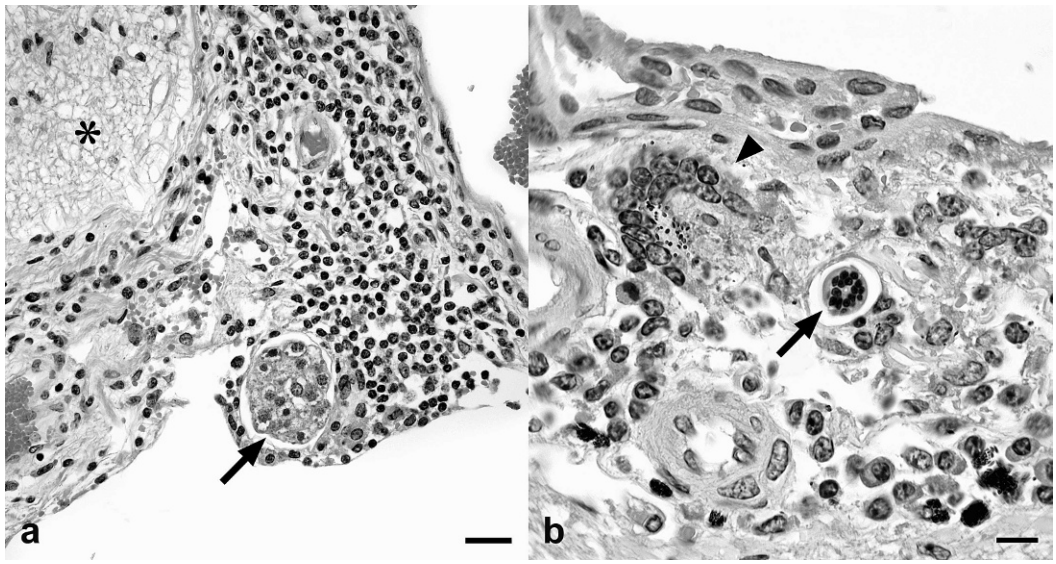

FIGURE 1. Sections of brain including leptomeninges from the affected sika deer: (a) The leptomeninges are greatly thickened by lymphocytes that surround an embryonated egg (arrow). The adjacent neuropil (asterisk) is rarefied due to edema fluid. H&E stain. Bar=25 μm. (b) A nematode larva (arrow) in the meninges is surrounded by inflammatory cells including plasma cells, lymphocytes, macrophages, and a single multinucleate giant cell (arrowhead). H&E stain. Bar=10 μm.

(pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 250 μM deoxynucleotide triphosphates, 0.5 μM of each primer, and 1 U of *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA) in a 25-μl reaction. Cycling parameters were: denaturation at 94 C for 3 min, followed by 40 cycles of denaturation at 94 C for 1 min, annealing at 60 C for 1 min, and extension at 72 C for 1 min. For the DNA extraction and PCR reactions, negative water controls were included to detect contamination. Reaction products were examined by electrophoresis in a 1% agarose gel and amplicons were excised and purified using a QIAquick® Gel Extraction kit (Qiagen) according to the manufacturer's instructions. Extracted DNA was cloned using pCR®4-TOPO® plasmid (Invitrogen, Carlsbad, California, USA) according to the manufacture's instructions. Sequencing of the plasmid was performed using primers T3 and T7 at the Integrated Biotechnology Laboratories at the University of Georgia using an Applied Biosystems Inc., 3100 Genetic Analyzer (Foster City, California).

The deer was classified as an adult based on size and molar wear (Takahashi et al., 1999). Gross lesions were not apparent and the cerebellum was negative for rabies by FA testing. The leptomeninges were moderately to severely thickened by variable numbers of lymphocytes and plasmas cells with fewer neutrophils, eosinophils, and macrophages. Small numbers of nematode larvae and eggs were scattered throughout the inflamed portions of the meninges (Fig. 1a, b). Rare foci contained multinucleate giant cells and variable amounts of hemosiderin usually associated with nematode eggs or larvae and some had engulfed eggs or larvae (Fig. 1b). The neuropil of the cerebrum, cerebellum, and brain stem contained areas of necrosis and inflammation with similar infiltrates as seen in the meninges. Many lymphocytes and plasma cells surrounded the vessels in the cerebrum, cerebellum, and brainstem. Three cross-sections of a nematode were present in a section of the cranial cerebrum. They were from 170 μm to 245 μm in diameter

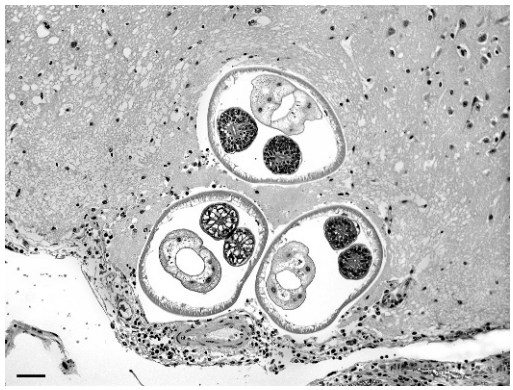

FIGURE 2. Section of brain containing three transverse sections of a large nematode consistent with *P. tenuis*. A small number of lymphocytes infiltrate the adjacent meninges and superficial aspects of the neuropil. H&E stain. Bar=50 μm.

and had a thin cuticle, polymyarian coelomyarian musculature, accessory hypodermal chords, and large multinucleate intestinal cells with a low microvillus border and no greater than two cells per cross section (Fig. 2). The connective tissue surrounding the optic nerves contained a few foci expanded by infiltrates of lymphocyte and plasma cells. The skeletal muscle surrounding the nerves contained areas of necrosis with moderate numbers of lymphocytes, plasma cells, and eosinophils. Scattered within the necrotic areas were several emboli containing larvated and embryonated eggs as previously described. The pulmonary interstitium was mildly thickened by few to moderate numbers of lymphocytes and plasma cells with fewer neutrophils. Nucleotide sequence analysis of the 228-bp PCR product (GenBank® accession No. GU122924) revealed a 99% identity to *P. tenuis* and a 96% identity to *P. andersoni* as compared to the Genbank® database accession number sequences AF504029 and EF173711, respectively.

The histologic characteristics of this nematode including the musculature, hypodermal chords, and large multinucleate intestinal cells are consistent with the superfamily Metastrongyloidea (Gardiner and Poynton, 1999; Anderson, 2000). Both the diameter and the anatomic location of this nematode are consistent with *Parelaphostrongylus tenuis* and *Elaphostrongylus rangiferi* (Lankester, 2001). Only *P. tenuis* is known to occur in the area where this deer was collected (Lankester, 2001) and the nematode was identified as *P. tenuis* based on the ITS-2 rRNA sequence. White-tailed deer are the definitive host of *P. tenuis* and terrestrial slugs and snails are required intermediate hosts. Animals are infected by ingestion of snails or slugs when feeding. *Parelaphostrongylus tenuis*–associated morbidity and mortality has been reported in a number of cervids including elk (*Cervus elaphus*), moose (*Alces alces*), and fallow deer (*Dama dama*; Anderson, 2000; Lankester, 2001), but to our knowledge, this is the first report of *P. tenuis* in a sika deer.

Sika deer are native to Japan, Manchuria, Taiwan, Korea, and adjacent China; however, they have been introduced into several locations in the United States including the Eastern Shore of Maryland, Assateague Island, Kansas, Oklahoma, Wisconsin, and Texas (Whitaker and Hamilton, 2003). The Maryland population (*C. nippon yakushimae*) was introduced from the Yakushima Island, Japan, in the early 1900s by private individuals. Genetic research indicates that no more than six individuals comprised the initial population that was released on James Island (B. Eyler, pers. comm.). Current population size in Maryland is estimated at 5,000 animals with the primary populations located in Dorchester County and on Assateague Island; however, the population is expanding into the wetland habitats of the lower Eastern Shore of Maryland (Eyler, pers. comm.). The Maryland population is currently managed by hunting with the goal of maintaining current population levels. It is unknown what impact *P. tenuis* will have on sika deer populations. Several anecdotal reports of sika deer appearing blind or exhibiting neurologic deficiencies have been reported recently from the area where this deer originated.

Eggs and larvae were found in the connective tissue and skeletal muscle surrounding the optic nerve, but not in the lungs of the deer described here. It is unknown if the lack of eggs and larvae in the lungs is due to a short duration of the infection or if a species barrier exists in sika deer inhibiting parasite development. Elk have been shown to be competent hosts for *P. tenuis* development and larval shedding (Lankester, 2001). It would be useful to determine if sika deer could support *P. tenuis* larvae development and, thus, potentially introduce the parasite into populations of white-tailed deer that are currently free of the parasite.

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