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Authors: Pybus, M. J., Groom, S., and Samuel, W. M.

Source: Journal of Wildlife Diseases, 32(4) : 614-618

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-32.4.614

Journal of Wildlife Diseases, 32(4), 1996, pp. 614–618

MENINGEAL WORM IN EXPERIMENTALLY-INFECTED BIGHORN AND DOMESTIC SHEEP

M. J. Pybus,[1] S. Groom,[2] and W. M. Samuel[3]

[1] Alberta Fish and Wildlife Services, 6909-116 Street, Edmonton, Alberta T6H 4P2, Canada
[2] Alberta Agriculture, 6909-116 Street, Edmonton, Alberta T6H 4P2, Canada
[3] Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

ABSTRACT: In the first (July 1989) of two experiments, each of three bighorn sheep (*Ovis canadensis*) and three domestic sheep, respectively, was exposed to 25, 150, or 300 infective third-stage larvae (L3) of the meningeal worm, *Parelaphostrongylus tenuis*. Two bighorn sheep had temporary mild paresis and lumbar weakness; one developed paralysis and died suddenly 32 days after exposure. Adult *P. tenuis* were found deep within the brain and spinal cord of the one latter sheep. A generalized inflammatory response, characterized by subdural lymphoid aggregations adjacent to spinal nerve roots, was seen in the spinal cord of most domestic and bighorn sheep. In the second experiment (September 1990), each of six domestic sheep lambs and five white-tailed deer (*Odocoileus virginianus*) fawns was exposed to a single dose of 15 to 125 L3 of meningeal worm. Clinical signs were seen in only one sheep; it was dull and depressed. No worms were found in this sheep. One dead adult meningeal worm was found on the brain of another sheep. First-stage larvae and adult meningeal worms were found in all deer.

Key words: Bighorn sheep, domestic sheep, meningeal worm, experimental study, *Parelaphostrongylus tenuis*, *Ovis canadensis*.

INTRODUCTION

Sporadic cases of fatal neurologic disease in sheep have been reported in eastern and northcentral United States (Jortner et al., 1985; O'Brien et al., 1986), despite the widespread occurrence of meningeal worm and relatively high densities of infected white-tailed deer (*Odocoileus virginianus*) throughout the region. Morbidity in infected flocks has ranged from 2% (Alden et al., 1975) to 59% (Jortner et al., 1985) and spontaneous recovery from clinical signs often has been observed (Alden et al., 1975). Adult nematodes were seen in the spinal tissue of most sheep with fatal infections. Few sheep have been infected experimentally with meningeal worm (Anderson and Strelive, 1966) and only those that received high numbers of parasites died.

There are no reports of infections of meningeal worm in bighorn sheep, *Ovis canadensis*. Free-ranging bighorn sheep are found only in western North America, where meningeal worm is not known to occur (Anderson and Prestwood, 1981), but captive bighorn sheep in eastern North America may be exposed in zoo or animal parks (Nichols et al., 1986). It is not known whether bighorn sheep are, like domestic sheep (Anderson and Strelive, 1966), resistant to infection with meningeal worm. In this study, we evaluated the effects of meningeal worm in bighorn and domestic sheep.

MATERIALS AND METHODS

In the first of two experiments, each animal in three pairs of bighorn and domestic sheep was given a single dose of 25, 150, or 300 infective third-stage larvae (L3) on 17 July 1989, while three control domestic sheep were not given larvae. In the second experiment, six domestic sheep were given single doses of 15, 25, 35, 55, 75, or 125 L3, respectively, and five control white-tailed deer received 15, 25, 35, 75, or 125 L3, respectively, on 12 September 1990. All L3 were from the same pooled sample and thus infections in deer served as controls on the viability and identity of larvae. As soon as patency was established, deer were killed with an intravenous injection of 100 mg/kg sodium pentobarbital (Euthanyl®, M.T.C. Pharmaceuticals, Mississauga, Ontario, Canada) following immobilization with 0.5 mg/kg xylazine hydrochloride (Rompun®, Haver-Lockhart Laboratories, Shaunee, Kansas, USA).

The 12 domestic sheep were 4-mo old and of mixed ancestry (primarily Suffolk). The three bighorn sheep, originally captured as lambs in the foothills region of Alberta, Canada,

(52°25′N, 115°45′W) were 4-yr old and had not received anthelmintics for at least 12 mo prior to the experiment. Five white-tailed deer fawns, originally collected as orphans from free-ranging deer from various regions of Alberta and reared using techniques of Pybus (1983), were weaned at approximately 3-mo of age. They, along with the domestic and bighorn sheep, were fed alfalfa pellets and dried hay ad libitum. All animals were held in individual pens (2 × 5 m) with cement floors. Domestic sheep and deer were held at the University of Alberta Research Station, near Ellerslie, Alberta, while bighorn sheep were held at the Alberta Agriculture Provincial Laboratory in Edmonton. Bedding from all pens was burned.

Meningeal worm larvae from a known source of infected white-tailed deer (Samuel et al., 1992) were used to infect laboratory-reared snails (*Triodopsis multilineata*). After at least 40 days, infective larvae were collected following pepsin-HCl digest of the snails (Prestwood, 1972). Larvae were kept in a mixture of digest solution and saline (approximately 30 C) and immediately transported to the animal-holding facilities.

Animals were immobilized as follows: bighorn sheep were immobilized with 200 to 300 mg of xylazine administered intramuscularly with a spring-powered jabstick; domestic sheep were restrained manually in a sternal or standing position; and fawns were restrained manually in a standing position. Once immobilized, each animal received a known dose of infective larvae administered via stomach tube into the rumen. At least 100 cc of warm physiological saline was then flushed through the tube and into the rumen. Glassware and equipment used to transport larvae or introduce them into the sheep or deer were cleaned thoroughly in hot water between exposures and later examined for remaining larvae; none was found.

All animals were observed immediately after exposure for signs of regurgitation. Subsequently, each animal was observed for 30 to 60 min each day for changes in behavior; in particular, we noted any changes in stance, gait, and body placement. Observations were intensified if clinical signs were noted in any animal. Beginning 60 to 66 days postexposure (dpe), fecal samples from each animal were examined daily for first-stage larvae using a modified Baermann technique (Welch et al., 1991) and methods as described in Samuel et al. (1992). Particular attention was given to avoiding contamination in the laboratory; separate glassware was used for samples from each individual.

All animals were killed as per the white-tailed deer. One bighorn sheep was eviscerated and stored at −20 C prior to examination; all other animals were examined fresh. Detailed necropsy procedures (Samuel et al., 1992) were used to evaluate each carcass, with particular attention to the central nervous system (CNS). Briefly, the brain was removed, sliced, and then teased apart at 6× magnification. The venous sinuses within the dura mater (transverse, sagittal, and cavernous sinuses) were opened. The spinal cord was removed, cut into sections, the meninges were examined and removed, and the underlying nervous tissue was teased apart at 6× magnification. All exposed surfaces of the cranial cavity and vertebral canal were examined at 6× magnification. Samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 μm thickness, and stained with hematoxylin and eosin.

Current guidelines of the Canadian Council of Animal Care (1984) were followed for all aspects of the study.

RESULTS

In the first experiment, progressive neurologic signs were seen in all bighorn sheep but not in domestic sheep. Transient minor ataxia, mild posterior bilateral paresis, and weakness in hocks were seen in two bighorn sheep, receiving 25 L3 and 150 L3, respectively. The bighorn sheep given 300 L3 had moderate ataxia, depression, and weakness in the hind quarters at 28 and 29 dpe, generalized ataxia and reluctance to stand at 30 dpe, lethargy and generalized loss of control over limbs during attempts to rise at 31 dpe, and recumbency and generalized paralysis at 32 dpe. The bighorn sheep given 25, 150 or 300 L3s were killed 133, 129, and 32 dpe, respectively, and the domestic sheep were killed 121, 106, and 36 dpe, respectively.

Gross and histologic lesions were variable. General mild meningitis and focal leucoencephalomalacia with occasional subdural lymph nodules associated with peripheral nerves were seen in two bighorn sheep, receiving 25 L3 and 150 L3, respectively. Mild meningitis was seen in one domestic sheep receiving 25 L3. There were numerous small (3 to 5 mm) firm nodules throughout the abdominal cavity, particularly the omentum; a few shallow pitted scars on the ventral surface of the liver; and small (1 to 2 mm) clear

fluid-filled blisters scattered over the pleura in both sheep that received 300 L3. Remnants of a nematode larva 40 μm in diameter were seen in one nodule. This domestic sheep also had focal hemorrhage in the neuropile, mild meningitis and malacia in the spinal cord, as well as multifocal encephalomalacia in the dorsal medulla and cerebral gray matter. Freezing artifacts prevented complete evaluation of lesions in the brain and spinal cord of the bighorn that received 300 L3; however, 13 (4.3% of dose) small non-gravid adult meningeal worms were found in the anterior medulla and throughout the spinal cord from the second cervical to the third sacral vertebra. Nine nematodes were in the white matter, three in the gray matter, and one was found in the subdural space at the third lumbar vertebra. All worms were coiled. Five female and six male worms, as well as pieces of worms, were found.

The three unexposed domestic sheep in the first experiment were killed 48, 132, or 142 days after the experimental sheep were exposed to L3. No clinical signs or lesions were seen in these sheep.

In the second experiment, only the domestic sheep given 125 L3 had clinical signs. It was dull and listless from 41 dpe until 124 dpe. All sheep were killed 124 to 138 dpe. Few gross and histologic lesions were seen. A mild chronic leucoencephalomalacia was seen in the brain of sheep given 55, 75, and 125 L3, respectively. One dead adult meningeal worm was found subdural in an accumulation of viscid yellow to orange exudate in the frontal region of the left cerebral hemisphere of the sheep that received 15 L3. Subdural lymph nodules along the spinal cord, occasional hemorrhage in the spinal gray matter, and lymphoid nodules with central mineralized necrotic debris in the cranial dura also were seen in the latter sheep. Dorsal-spined larvae were not found in the feces of any sheep, but were found in feces of all five deer (mean ± SD prepatent period was 94 ± 5 days). Adult *P. tenuis*, found in all five deer, were the only nematodes recovered.

DISCUSSION

This is the first study in which bighorn sheep were evaluated as hosts for meningeal worm. Their response to this parasite generally did not differ from that of domestic sheep; both species appeared to resist light infections, but died if exposed to relatively high numbers of meningeal worm (Anderson and Strelive, 1966). This is consistent with *P. tenuis* infections in other mammals, although the dose at which mortality occurs differs among species. Domestic sheep given 500 or more L3 died (Anderson and Strelive, 1966), but most of those given 300 or less L3 did not (Anderson and Strelive, 1966; this study). All elk (*Cervus elaphus*) given 125 or more L3 died, but most given 75 or less L3 survived (Samuel et al., 1992). All fallow deer (*Dama dama*) exposed to 25 or more L3 died (Pybus et al., 1992), as did most llamas (*Lama glama*) given five or more L3 (Foreyt et al., 1991; Rickard et al., 1994). The wide variation in response likely reflects inherent differences in genetic makeup (Wakelin, 1976) as they relate to the number of larvae that reach the CNS and the specific CNS damage caused by the parasite (Anderson and Strelive, 1966; Poynter, 1966; Handeland, 1991).

The specific response of sheep to meningeal worm appears to differ from that of most cervids. Although fallow deer have some resistance to low numbers of meningeal worm larvae (Davidson et al., 1985), other cervids appear unable to limit the number of larvae which reach the CNS and thus, mortality can occur at relatively low doses. Individuals that do not die, often develop patent infections. However, in both bighorn and domestic sheep, migrating larvae generally were killed before they reached the CNS and fatal damage was avoided. A similar situation may occur in domestic sheep exposed to *Elaphostrongy-*

lus spp., close relatives of meningeal worm (Handeland, 1991). Thus, although most sheep survive exposure to meningeal worm, it is unlikely that they develop patent infections.

Due to disparate natural distributions, bighorn sheep held in captivity in eastern North America may be the only individuals likely to encounter meningeal worm (Nichols et al., 1986). Even if meningeal worm became established in western regions, mortality of bighorn sheep would be expected only if the sheep were exposed to relatively high numbers of larvae and in areas where free-ranging white-tailed deer and bighorn sheep were sympatric.

ACKNOWLEDGMENTS

The authors heartily thank C. J. Wilke and D. A. Welch for the many hours spent on this project. Comments on the manuscript by D. A. Welch were particularly helpful. C. M. Gerla, S. C. Kerswell (Bioscience Animal Services, University of Alberta) and T. Ewaschuk assisted in raising and maintaining the domestic sheep and white-tailed deer. W. D. Wishart and R. McClymont (Alberta Fish and Wildlife) provided the bighorn sheep. D. Onderka and A. Perry (Alberta Agriculture) provided assistance in interpreting clinical and histopathology results. Personnel of Alberta Agriculture, particularly T. Church, provided encouragement, laboratory services and logistical support. This project was funded by an Alberta Agriculture (Farming for the Future) grant to WMS.

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Received for publication 23 November 1993.