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# Bighorn Sheep, a New Host Record for *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae)

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ABSTRACT: Larval nematodes with a dorsal spine on the tail were recovered from fecal samples of California bighorn sheep (*Ovis canadensis californiana*) in northeastern Washington State, USA. The identity of these dorsal-spined larvae (DSL) was established by single-strand conformation polymorphism (SSCP) analyses of a partial fragment of the first internal transcribed spacer of the ribosomal DNA. The SSCP profiles of individual DSL from bighorn sheep were compared with those of DSL of five protostrongylid species (*Parelaphostrongylus andersoni*, *P odocoilei*, *P. tenuis*, *Elaphostrongylus rangiferi*, and *Muellerius capillaris*) but were identical to only those of *P. odocoilei*. This study represents the first confirmed identification of *P. odocoilei* in bighorn sheep.

*Key words:* Bighorn sheep, *Muellerius capillaris*, muscleworm, *Ovis canadensis*, *Parelaphostrongylus odocoilei*, Protostrongylidae, single-strand conformation polymorphism (SSCP), Washington State.

Four species of protostrongylid nematodes, *Protostrongylus stilesi*, *P. rushi*, *P. frosti* and *Muellerius capillaris*, have been reported in bighorn sheep, *Ovis canadensis* (Forrester, 1971; Demartini and Davies, 1977; Pybus and Shave, 1984; Spraker et al., 1984; Hoberg et al., 2002), although the status of *P. frosti* as a distinct species to *P. stilesi* has been disputed (Dougherty and Goble, 1946; Dikmans, 1957; Forrester, 1971). *Protostrongylus stilesi* and *M. capillaris* are known to seriously affect the health of bighorn sheep (Forrester, 1971; Demartini and Davies, 1977; Spraker et al., 1984; Monello et al., 2001). These parasites are an important contributing factor of mortality in cases of stress-lungworm-pneumonia complex, primarily by predisposing the lungs to secondary bacterial and viral infection (Forrester, 1971; Spraker et al., 1984; Monello et al., 2001). The detection of protostrongylid nematodes in hosts is often based on the recovery of first-stage larvae in fecal samples. First-stage larvae of *M. capillaris* differ from those of *Protostrongylus* spp. by bearing a dorsal spine on the tail. Other dorsal-spined larvae (DSL) morphologically similar to, but greater in length than, DSL of *M. capillaris* have also been recovered from free-ranging bighorn sheep in Alberta and British Columbia, Canada (Pybus and Shave, 1984). The identification of these larvae to species was not established. Foreyt et al. (1996) also detected DSL in four of 230 fecal samples taken from a bighorn sheep herd on Hall Mountain in northeastern Washington, USA. They suggested that these DSL may belong to the protostrongylid genus *Parelaphostrongylus* (subfamily Elaphostrongylinae), but the presence of nematodes of this genus in bighorn sheep has yet to be confirmed.

The identification of protostrongylid DSL to the species or genus level based on geographic location and host species is not feasible because of the possibility of mixed infections and overlapping host distributions (Pybus and Samuel, 1981; Lankester and Fong, 1998). Moreover, as DSL of different elaphostrongyline species are morphologically and morphometrically similar (Lankester and Hauta, 1989; Gajadhar, et al., 1994), only molecular studies have been used effectively for identification of species (Gajadhar et al., 2000; Chilton et al., 2005; Jenkins et al., 2005a; Huby-Chilton et al., 2006). The



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DNA sequencing of the second internal transcribed spacer (ITS-2) rDNA has been used in species identification and for determining the geographic distribution and host range of *P. odocoilei* (see Jenkins et al., 2005a). However, the use of such an approach for the identification of large numbers of individual nematodes is impractical, relatively time consuming, and more expensive than some other molecular techniques, such as single-strand conformation polymorphism (SSCP) (see Gasser and Chilton, 2001). Recently, a diagnostic method was established, based on SSCP analysis of the first internal transcribed spacer (ITS-1) rDNA, for the accurate identification of single DSL from fecal samples of North American cervids (Huby-Chilton et al., 2006). This mutation scanning approach provided a reliable method to differentiate *Parelaphostrongylus andersoni* from *Elaphostrongylus rangiferi* in mixed infections of woodland caribou (*Rangifer tarandus caribou*) in Newfoundland, Canada. Single-strand conformation polymorphism was also used to identify DSL in white-tailed deer (*Odocoileus virginianus*) and black-tailed deer (*O. hemionus columbianus*) originating from different localities in North America. The aim of the present study was to use SSCP to identify, to the species-level, DSL recovered from bighorn sheep in northeastern Washington, USA.

Fresh fecal samples were collected from 16 California bighorn sheep (*O. c. californiana*) on Vulcan Mountain (48°59′N, 118°39′W), in northeastern Washington, USA. The sheep were observed voiding the samples just before collection. The feces of four of the 16 sheep contained DSL. These DSL were extracted from the feces using a modified Baermann beaker technique, as described previously (Foreyt, 2001), and stored in 70% ethanol. Wet mounts of the DSL were prepared in water and examined with a compound microscope to confirm the presence of a dorsal spine. For the molecular analyses, 10 ethanol-fixed DSL were washed three times in sterile nanopure water. Genomic (g) DNA was isolated from individual DSL by sodium dodecyl-sulfate/proteinase K treatment and then column-purified using the Wizard™ DNA CleanUp kit (Promega, Madison, Wisconsin, USA) (Chilton et al., 2005). A partial fragment (317–336 bp) of the ITS-1 rDNA and 5′ flanking region (76 bp of the 18S rRNA gene) was amplified by PCR from 2 µl of gDNA using the oligonucleotide primers NC16 (forward, 5′-AGT TCA ATC GCA ATG GCT T-3′) and R1 (reverse, 5′-GCA TTC TAG CAA TGC TCA TT-3′) and the conditions described by Huby-Chilton et al. (2006). Each amplicon was subjected to electrophoresis in a 1.5% (w/v) agarose-TBE (65 mM Tris-HCl, 22.5 mM boric acid, 1.25 mM EDTA, pH 9) gel. The SSCP analysis was performed on single larvae as described previously (Huby-Chilton et al., 2006). In brief, NC16-R1 amplicons were mixed with an equivalent volume of loading buffer (Elchrom Scientific, Cham, Switzerland), denatured at 94 C, then snap-cooled on a freeze block (−20 C), before loading into the wells of precast GMA™ Wide Mini S-50 gels (Elchrom Scientific). Gels were run at 72 V for 17 hr at a controlled, constant temperature of 7.4 C, in a horizontal SEA 2000® (Elchrom Scientific) electrophoretic apparatus connected to a circulating water bath. After electrophoresis, SSCP gels were stained in ethidium bromide for 15 min and then destained in $H_2O$ for 10 min before examination with ultraviolet (UV) transillumination using an AlphaImager 2000 gel documentation system (Alpha Innotech Corporation, San Leandro, California, USA). The identity of each of the 10 DSL assayed was determined by comparing their pITS-1 SSCP profiles with the profiles of DSL of *M. capillaris*, *P. odocoilei*, *P. tenuis*, *P. andersoni*, and *E. rangiferi* obtained from hosts known to be infected with only one species of protostrongylid nematode. The identity of the reference DSL was based on morpholog-

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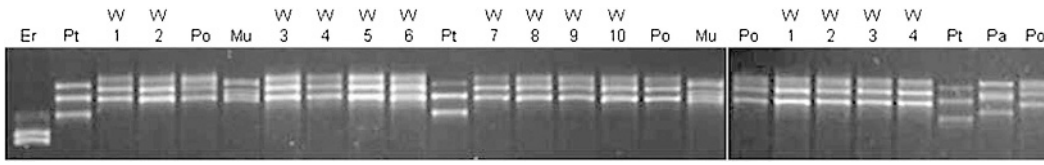


FIGURE 1. Comparison of the single-strand conformation polymorphism (SSCP) profiles of the partial first internal transcribed spacer (pITS-1) rDNA for individual dorsal-spined larvae from bighorn sheep of Washington, USA (W1 to W10) with those of known (control) samples: *Elaphostrongylus rangiferi* (Er), *Muellerius capillaris* (Mu), *Parelaphostrongylus andersoni* (Pa), *P. tenuis* (Pt), and *P. odocoilei* (Po).

ical examination of adult male worms recovered from host necropsies (Gajadhar et al., 2000; Huby-Chilton et al., 2006).

The partial ITS-1 amplicons of all 10 DSL from the bighorn sheep consisted of a single band of approximately 400 bp in size (data not shown). The results of SSCP analyses conducted on the amplicons of each of the 10 DSL from bighorn sheep and representative DSL of five species of protostrongylid nematodes are shown in Figure 1. The SSCP profiles of the known positive controls had either three bands (for *E. rangiferi*, *P. odocoilei*, and *P. tenuis*) or four bands (for *M. capillaris* and *P. andersoni*). The SSCP profiles of the *Parelaphostrongylus* spp. and *E. rangiferi* were identical to those determined previously (Huby-Chilton et al., 2006). *Muellerius capillaris*, which is known to occur in bighorn sheep (Pybus and Shave, 1984), displayed a unique SSCP profile when compared with each species of *Parelaphostrongylus* and *Elaphostrongylus*. This ability to distinguish individual *M. capillaris* larvae from those of the elaphostrongylines enhances the utility of this molecular method in the delineation of the different species of DSL occurring in North America. For instance, *M. capillaris* has been identified from bighorn sheep in South Dakota, USA, based on the morphology of adults removed from lung tissue, whereas other reports of this species in bighorn sheep have been based on the presence of DSL in feces (Pybus and Shave, 1984). In the latter cases, such identifications are not conclusive because several species of protostrongylid produce DSL. The SSCP analyses of the partial ITS-1 could be used effectively in such situations to verify the presence of *M. capillaris* DSL in bighorn sheep.

The partial ITS-1 amplicons of each DSL from bighorn sheep from northeastern Washington, USA, revealed the same three-banded SSCP profile (Fig. 1), which was identical only to that of the *P. odocoilei* control. Thus, these unknown DSL were identified as the muscleworm, *P. odocoilei*, which represents the first confirmed identification of this species in bighorn sheep. *Parelaphostrongylus odocoilei* has been previously reported in cervids, such as the mule deer (*O. hemionus hemionus*), black-tailed deer (*O. h. columbianus*) (Hobmaier and Hobmaier, 1934; Brunetti, 1969; Platt and Samuel, 1978; Pybus et al., 1984; Jenkins et al., 2005a; Huby-Chilton et al., 2006), and woodland caribou (*Rangifer tarandus caribou*) (Gray and Samuel, 1986; Jenkins et al., 2005a) and in mountain goats (*Oreamnus americanus*) (Pybus et al. 1984; Jenkins et al., 2005a). Recently, *P. odocoilei* has also been found in thinhorn sheep (*Ovis dalli*) from the Northwest Territories (Kutz et al., 2001, Jenkins et al., 2005a), Yukon Territory, and British Columbia, Canada, and Alaska, USA (Jenkins et al., 2005a), demonstrating a larger geographic distribution and a broader range of hosts than previously thought for this species.

Jenkins et al. (2005a) proposed that many bighorn sheep populations are likely

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to be exposed to *P. odocoilei* because of three factors: 1) *P. odocoilei* has a broad host range; 2) there are similarities in the endemic parasite fauna of bighorn sheep, thinhorn sheep, and mountain goats (Hoberg et al., 2001); and 3) the ranges of bighorn sheep overlap those of infected mule deer and mountain goats. However, they found no evidence of DSL in 31 fecal samples from Rocky Mountain bighorn sheep (*O. canadensis canadensis*) populations at Radium (Kootenay Mountains, British Columbia) and at Cardinal River (Rocky Mountains, Alberta), Canada, even though fecal samples of four mule deer at Cardinal River, Canada, were found to contain *P. odocoilei*. DSL, identified as *P. odocoilei* using DNA sequencing of the ITS-2 rDNA, were recovered from one of 412 fecal samples from a California bighorn sheep (*O. c. californiana*) population at Fraser River (Lillooet Mountains, British Columbia, Canada) (Jenkins et al., 2005a). These were dismissed as contamination from feces of sympatric mule deer infected with *P. odocoilei*, although *Protostrongylus* sp. larvae and eggs of gastrointestinal nematodes typical of bighorn sheep were present in the composite fecal sample (Jenkins et al., 2005a). Other studies have reported the presence of unidentified DSL in bighorn sheep in Alberta and British Columbia, Canada, and Montana, North Dakota, and Washington, USA (Pybus and Shave, 1984; Foreyt et al., 1996). The occurrence of *P. odocoilei* in some of these areas (e.g., Newhalem, Washington, USA, and Jasper National Park, Alberta, Canada) has been confirmed, based on morphologic examinations of adult worms, only in mule deer (Platt and Samuel, 1978) and mountain goats (Pybus et al., 1984). Although Foreyt et al. (1996) suggested that the unidentified DSL in bighorn sheep from northeastern Washington, USA, could possibly be *Parelaphostrongylus*, our study provides definitive evidence of the presence of *P. odocoilei* DSL in bighorn sheep. The recovery of *P. odocoilei* from bighorn sheep in northeastern Washington, USA, further increases the host range of this parasite species.

The prevalence of *P. odocoilei* in bighorn sheep is significantly lower than in other host species. Four of 16 (25%) fecal samples from bighorn sheep on Vulcan Mountain (northeastern Washington, USA) contained *P. odocoilei* (this study), whereas only four of 230 samples (2%) from bighorn sheep on Hall Mountain (northeastern Washington, USA) contained DSL suspected to be *Parelaphostrongylus* (Foreyt et al., 1996). The parasite may be absent in three bighorn sheep populations in British Columbia and Alberta, Canada (Jenkins et al., 2005a). This is in contrast to the high prevalence of *P. odocoilei* recorded previously in thinhorn sheep (66–100%), mule deer (80–100%), mountain goats (41–100%), woodland caribou (28–43%), and blacktailed deer (25–56%) (Kutz et al., 2001; Jenkins et al., 2005a). This suggests that bighorn sheep in northeastern Washington, USA, may represent an accidental host for this parasite.

Ungulates become infected with *P. odocoilei* following the accidental ingestion of gastropods containing third-stage larvae. *Parelaphostrongylus odocoilei* is commonly called the muscleworm because adults are associated with small veins in connective tissue between bundles of skeletal muscle (Pybus and Samuel, 1984a). The precise migratory route taken by developing worms to the muscles is uncertain but is believed not to involve the central nervous system (CNS) (Platt and Samuel, 1978). However, there is some indirect, recent evidence suggesting that *P. odocoilei* migrate through the CNS to the musculature of thinhorn sheep (Jenkins et al., 2005b). The eggs laid by females travel in the venous blood to the lungs where they hatch. The first-stage larvae move into the alveolar spaces, then up the bronchial elevator, where they are swallowed and passed in feces to the external environment, where they infect

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a terrestrial gastropod (Lankester, 2001). The pathogenic effects of *P. odocoilei* on mule deer and white-tailed deer have been demonstrated to vary considerably in experimental infections (Pybus and Samuel, 1984*a*), which may be a reflection of differences in their relative susceptibility to infection and the number of female worms maturing in muscles (Lankester, 2001). Hemorrhagic myositis and interstitial pneumonia have been reported in mule deer (Pybus and Samuel, 1984*b*; Pybus et al., 1984), whereas *P. odocoilei* in thinhorn sheep may cause neurologic and respiratory disease (Kutz et al., 2001; Jenkins et al., 2005*b*). The death of mountain goats in Washington, USA, and Alberta, Canada, has also been attributed to infection with *P. odocoilei* (Pybus et al., 1984). Although *P. odocoilei* may significantly affect the health of some ungulates, the effect of this parasite species on bighorn sheep is currently unknown. Further study is required because *P. odocoilei* may be important as a predisposing factor in the pneumonia-complex of bighorn sheep.

In conclusion, SSCP of the ITS-1 rDNA provided a rapid and reliable method for the detection of *P. odocoilei* in bighorn sheep in northeastern Washington, USA. It also confirmed the value of this method as a diagnostic tool for the identification of protostrongylid species with DSL in previously recognized or new hosts in North America. The geographic and host ranges of these parasite species could be further investigated using SSCP, especially where overlapping distributions of wild and domestic populations of potential host species occur. This is particularly important because of the potential pathogenic effects posed by these parasites.

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