

PARELAPHOSTRONGYLUS ODOCOILEI IN COLUMBIAN BLACK-TAILED DEER FROM OREGON

Authors: Mortenson, Jack A., Abrams, Arthur, Rosenthal, Benjamin M., Dunams, Detiger, Hoberg, Eric P., et al.

Source: Journal of Wildlife Diseases, 42(3) : 527-535

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-42.3.527>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

PARELAPHOSTRONGYLUS ODOCOILEI IN COLUMBIAN BLACK-TAILED DEER FROM OREGON

Jack A. Mortenson,^{1,5} Arthur Abrams,² Benjamin M. Rosenthal,² Detiger Dunams,² Eric P. Hoberg,² Robert J. Bildfell,³ and Richard L. Green⁴

¹ United States Department of Agriculture, Veterinary Services, 530 Center St. NE, Suite 335, Salem, Oregon 97301, USA

² United States National Parasite Collection and Animal Parasitic Disease Laboratory, United States Department of Agriculture, Agricultural Research Service, BARC East No. 1180, Beltsville, Maryland 20705

³ Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97330, USA

⁴ Oregon Department of Fish and Wildlife, 7118 NE Vandenberg Ave., Corvallis, Oregon 97330, USA

⁵ Corresponding author (email: jack.a.mortenson@usda.gov)

ABSTRACT: Documenting the occurrence of *Parelaphostrongylus odocoilei* has historically relied on the morphological examination of adult worms collected from the skeletal muscle of definitive hosts, including deer. Recent advances in the knowledge of protostrongylid genetic sequences now permit larvae to be identified. Dorsal-spined larvae (DSLs) collected in 2003–2004 from the lung and feces of six Columbian black-tailed deer (*Odocoileus hemionus columbianus*) from Oregon were characterized genetically. The sequences from unknown DSLs were compared to those from morphologically validated adults and larvae of *P. odocoilei* at both the second internal transcribed spacer (ITS-2) of ribosomal DNA and the mitochondrial cytochrome oxidase II gene. We provide the first unequivocal identification of *P. odocoilei* in Columbian black-tailed deer from Oregon. The broader geographic distribution, prevalence, and pathology of *P. odocoilei* are not known in populations of Oregon deer.

Key words: Columbian black-tailed deer, COX-II, ITS-2, muscle worm, *Odocoileus hemionus columbianus*, *Parelaphostrongylus odocoilei*.

INTRODUCTION

Recognized as a parasite of the skeletal musculature, the nematode *Parelaphostrongylus odocoilei* has been historically documented in Columbian black-tailed deer (*Odocoileus hemionus columbianus*) from California and British Columbia (Hobmaier and Hobmaier, 1934; Pybus et al., 1984) and in mule deer (*Odocoileus hemionus hemionus*) from Alberta, Canada (Platt and Samuel, 1978; Samuel et al., 1985). Other hosts identified include woodland caribou (*Rangifer tarandus caribou*) (Gray and Samuel, 1986); mountain goat (*Oreamnos americana*) (Pybus et al., 1984); and thinhorn sheep, including Dall's sheep (*Ovis dalli dalli*) and Stone's sheep (*O. dalli stonei*) (Kutz et al., 2001; Jenkins, 2005). More recently, Jenkins et al. (2005) documented *P. odocoilei* based on molecular methods and assessment of larval sequences in all of the above-mentioned host species across a broad

range from California to Alaska and the Northwest Territories (NT), Canada.

Efforts were made in 2003–2004 to determine whether this parasite was present in Columbian black-tailed deer in western Oregon after observing verminous pneumonia and dorsal-spined larvae (DSLs) in lung tissue and feces of necropsied deer (Bildfell et al., 2004). Until recently, differentiating among species of *Parelaphostrongylus* has required collecting and examining adult worms from skeletal muscle, because the DSLs of *Parelaphostrongylus* and some other protostrongyles in North American ungulates cannot be unequivocally identified based on morphological characters (Platt and Samuel, 1978; Carreno and Lankester, 1993; Kutz et al., 2001; Jenkins et al., 2005).

Differences in the second internal transcribed spacer (ITS-2) of ribosomal DNA have been used to identify many nematode parasites (Gasser and Monti,

1997; Criscione et al., 2005), including specimens of DSLs among elaphostrongylines and other protostrongyles (Gajadhar et al., 2000). Restriction digestion of this locus, however, has proven to be equivocal, and it has now been demonstrated that direct sequence comparisons are required to differentiate among species of *Parelaphostrongylus* if the ITS-2 is the basis for identification (Jenkins et al., 2005). The paucity of variation in that locus, and the occurrence of distinct paralogs within individual larvae, may complicate attempts to differentiate closely related species of elaphostrongylines. Direct sequencing of more phylogenetically informative loci such as mitochondrial cytochrome oxidase may be preferable for exploring population level relationships (Jenkins et al., 2005).

Sequences for the ITS-2 of six protostrongylid species are now available in GenBank (Gajadhar et al., 2000; Junnila, 2002; Jenkins et al., 2005). Using methods developed by Jenkins et al. (2005), we compared sequences from morphologically validated adult specimens with otherwise unknown DSLs collected from wild Columbian black-tailed deer. We report the results of multilocus comparisons (nuclear ITS-2 and mitochondrial cytochrome oxidase II [COX-II]) leading to the first identification of *P. odocoilei* in Oregon, and a preliminary indication of genetic diversity associated with this nematode across its extensive range in western North America. Additionally, we provide the first mitochondrial COX-II sequences for the congeners *P. tenuis* and *P. andersoni* as an additional basis for comparison.

MATERIALS AND METHODS

Lung tissue and a minimum of 10 g of fecal material were removed from eight Columbian black-tailed deer found in Lincoln and Benton counties of western Oregon and submitted to the Oregon State University School of Veterinary Medicine Diagnostic Laboratory in 2003–2004. All sampled deer showed ad-

vanced clinical signs of hair-loss syndrome (Bender and Hall, 2004; Bildfell et al., 2004; Foreyt et al., 2004), with two being euthanized and six found dead. Larvae were collected by using a modified Baermann apparatus (Foreyt, 1997), with the lung tissue being homogenized to ensure better migration of first-stage larvae. After a 24-hr settling period, larvae were centrifuged to concentrate and decanted into tubes with 95% ethanol solution for storage for five samples, and three samples were held live in physiological saline. Samples were submitted to the US National Parasite Collection (USNPC) and the Animal Parasitic Disease Laboratory, Beltsville, Maryland, for analysis of DNA sequences. Dorsal-spined larvae of protostrongyles were subsampled from each host in which they were demonstrated by microscopic examination. Individual DSL from each host were selected and prepared for sequence analyses, and physical voucher specimens were deposited in the USNPC (Table 1). Initial identification was based on structure of the tail and size of the DSLs, generally consistent with species of *Parelaphostrongylus* (Lankester, 2001; Kutz et al., 2001; Jenkins et al., 2005). Specimens available for study varied in condition based on the timing of collections, methods of preservation and handling of materials subsequent to Baermann extraction. We did not always have live specimens on which to perform extractions and sequencing; variation in results and success in extraction may be attributable to these factors.

Specimens

Individual larvae from four hosts were characterized based on sequence comparisons. Larvae from two additional hosts were morphologically consistent with *P. odocoilei*, but they were not amenable to molecular analysis (dead and in poor condition). Sequences of the ITS-2 of nuclear rDNA of these larvae were compared with those derived from definitively identified adult specimens of *P. odocoilei* vouchered in the USNPC (from Dall's sheep, USNPC 94329, 94331, 94332, 94333, 94334), experimentally infected Stone's sheep (94891, 94892, 94893, 94894), and known sequences for *P. tenuis* and *P. andersoni* in GenBank. Additionally, comparisons were achieved with ITS-2 sequences from DSLs across the known range of *P. odocoilei* as summarized in an extensive geographic survey by Jenkins et al. (2005); these DSLs included those from Columbia black-tailed deer in northern California (Table 1).

TABLE 1. Samples of dorsal-spined larvae (DSLs) consistent with *Parelaphostrongylus odocoilei* in *Odocoileus hemionus columbianus* from Oregon and California based on morphology and sequences from the second internal transcribed spacer (ITS-2) and cytochrome oxidase II (COX-II) loci, and comparative materials from *P. odocoilei*, *P. tenuis*, and *P. andersoni*.

Host	USNPC	Locality ^a	Larvae At/Sq ITS-2	Larvae At/Sq COX-II
<i>Parelaphostrongylus odocoilei</i>				
Oregon				
OR-6635 ^b	95260 ^c	44°43'N, 123°55'W	5/1 ^d	10/5 ^d
OR-6684	95261	44°37'N, 123°59'W	10/0	10/4
OR-7524		44°39'N, 123°33'W	10/2	10/0
OR-8408	94882	44°38'N, 123°27'W	19/9	19/10 ^e
OR-8244	95263	44°23'N, 124°02'W	20/19	10/10 ^e
OR-5995	95262	44°30'N, 123°24'W	15/0	15/4 ^e
California (University of California, Hopland Research and Extension Center)				
CA-0127	94338	38°58'N, 123°07'W	10/1	10/4
CA-0105	94337	38°58'N, 123°07'W	10/1	10/5
CA-0113		38°58'N, 123°07'W	5/1	5/1
Northwest Territories (NT)				
	94329, 94333	Adult male and female with definitive morphological identification. ^f		
<i>Parelaphostrongylus tenuis</i> from <i>Odocoileus virginianus</i>				
		British Columbia/Minnesota ^g		
<i>Parelaphostrongylus andersoni</i> from <i>Rangifer tarandus groenlandicus</i>				
	94890	Beverly herd, NT, Canada ^f		

^a Geographic locality in degrees latitude and longitude.

^b Field collection number.

^c Accession number for physical vouchers in US National Parasite Collection.

^d Number of larvae studied (At = attempted; Sq = sequenced).

^e These sequences are partial (short) and were not used in tree construction, but they are consistent and do not refute an identification as *P. odocoilei*.

^f See Jenkins et al. (2005) for ITS-2 and Hoberg et al. (2005) for COX-II.

^g Sequence for ITS-2 from Gajadhar et al. (2000) and Junnila (2002). GenBank accession nos. AF504029 and AF504035; COX-II sequences represent this material.

Sequences for the mitochondrial COX-II locus were examined in the samples of larvae from Oregon. Comparisons at this locus are based on samples of identified DSLs and adults of *P. odocoilei* in Columbian black-tailed deer from northern California and adults in *O. d. dalli* from the Mackenzie Mountains, Canada, the latter specimens with vouchers previously deposited in the USNPC as accessions 94329 and 94333 (Hoberg et al., 2005; Jenkins et al., 2005). Furthermore, these specimens were compared with sequences of adult *P. tenuis* derived from white-tailed deer (*Odocoileus virginianus*) and larval *P. andersoni* from Barren-ground caribou (*Rangifer tarandus groenlandicus*) to establish an unequivocal basis for species identification (Table 1).

DNA extraction and amplification

The DNA from individual DSLs, including both live and preserved specimens, was extracted using a modification of the standard QIAGEN DNeasy protocol (QIAGEN, Valencia, California) by using two additional washes, and a 10-min incubation, before final elution. Polymerase chain reactions (PCRs) were performed to amplify ITS-2 and COX-II. To amplify ITS-2, primers NC1 (5'-ACG TCT GGT TCA GGG TTG TT-3') and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') were used (Gasser et al., 1993). To amplify COX-II, primers MTD16 (5'-ATT GGA CAT CAA TGA TAT TGA-3') and MTD18 (5'-CCA CAA ATT TCT GAA CAT TGA CCA-3') were used (Simon et al., 1994). Primers to amplify the second half of COX-II correspond to those

denoted as C2-J-3400 and C2-M-3661 in Simon et al. (1994) as originally specified by Liu and Beckenbach (1992). The 3' position of each primer corresponds to position 3400 and 3661 of the *Drosophila yakuba* mitochondrial genome. A second pair of primers also was developed from conserved sequences to aid in amplifying certain specimens: MTD16int (5'-TAT GAG TTT AGT GAT ATT CC-3') and MTD18int (5'-CTC AAA ATA CCT CTT ATA GC-3').

The standard protocol for Platinum High Fidelity Taq polymerase (Invitrogen, Carlsbad, California) was used for each 20- μ l PCR reaction. Each reaction was composed of 1 \times PCR buffer, 0.6 mM MgSO₄, 0.2 mM dNTP mixture, 0.5 μ M each primer, 0.25 units of Platinum High Fidelity Taq polymerase, and 2–2.5 μ l of template.

Amplification of ITS-2 used an initial 94 C denaturation for 3 min followed by 35–45 cycles of 94 C for 1 min, 55 C for 1 min, and 72 C for 2 min. Cycle parameters used for the COX-II consisted of an initial 3-min denaturation 94 C followed by 40–45 cycles of 30 sec at 94 C, 30 sec at 45–50 C, and 30 sec at 72 C. Each assay included a terminal extension step of 72 C for 10 min and was followed by cooling to 4 C. Each experiment included reactions containing no template, PCR (reagents only), and extraction negative controls to detect potential contamination. Reactions were analyzed by electrophoresis through a 1.4% agarose gel with ethidium bromide staining.

Sequencing

To prepare PCR products for direct fluorescent sequencing, 1.6 μ l of ExoSap-IT® (USB, Cleveland, Ohio) was added to 4 μ l of the PCR product to remove excess primers and dNTPs. Samples were then incubated at 37 C for 15 min and then heated to 80 C for 15 min. To complete the sequencing reaction, 4 μ l of BigDye® Terminator version 3.0 or 3.1 (Applied Biosystems, Foster City, California) and 3.2 pmol of primer were added before cycle sequencing. When possible, PCR products were sequenced in both directions by using the ITS-2 or the COX-II primers. Samples were then electrophoresed on an ABI 3100 or ABI 3730 capillary sequencer.

Data analyses

Sequence chromatograms were aligned and edited using Sequencer 4.1 software (GeneCodes Corp., Ann Arbor, Michigan). Aligned sequence chromatograms were inspected for the occurrence of polymorphic sites, and

consensus sequences were used in subsequent phylogenetic analyses, including homologous sequences from the congeners *P. tenuis* and *P. andersoni*. The relationships among all individual COX-II haplotypes were inferred by means of Neighbor-Joining phylogenetic reconstruction based on Kimura two-parameter genetic distances with PAUP* (Swofford, 2001). Because complete sequences were not available from each larval specimen, analyses were performed using both the complete deletion method, which bases all pairwise comparisons on the same subset of available data, as well as the pairwise deletion method, which uses all available comparative data from each sequence pair.

RESULTS

Dorsal-spined larvae were recovered from six of eight deer; other larvae present in the Baermann samples included *Dictyocaulus* cf. *eckerti*. The ITS-2 of all 29 larvae sequenced from four Columbian black-tailed deer from Oregon (OR-6635, OR-8408, OR-7524, and OR-8244) corresponded perfectly to that described by Jenkins et al. (2005) for *P. odocoilei* over the 270 base pairs (bp) available for comparison in all specimens. Perfect correspondence to full-length (571-bp) ITS-2 sequence of *P. odocoilei* also was observed for 12 larvae derived from host OR-8244, including the occurrence of “double traces” in three of five nucleotide positions previously reported to exemplify instances of nonuniformity among distinct copies in individual genomes (Jenkins et al., 2005). All ITS-2 sequences from Oregon are reported here for the first time.

Within the ITS-2, a mononucleotide polyadenosine repeat proved difficult to sequence in several of our specimens. This difficulty in sequencing was probably caused by variable numbers of adenosines in the population of amplified PCR product, but we do not know whether such length variants occur endogenously among the various nuclear copies of ITS-2, or instead, whether such variants arose de novo during the course of in vitro amplification. Nevertheless, the uniformi-

ty of the available ITS-2 sequences, outside this repeat region, permitted us to compare even those specimens with dual directional sequencing.

The total variation evident among COX-II sequences derived from these DSLs, and previously identified in two adult *P. odocoilei*, is illustrated in Table 2. Those larval sequences complete enough to permit comparison with at least 80% of the available adult sequences were subsequently used to reconstruct their relationships under the criterion of Minimum Evolution by using Kimura two-parameter distances (Fig. 1). Among these 12 larvae (one from CA-113, two each from CA-127 and OR 6684, and three each from CA-105 and OR- 6635), a total of 10 distinct haplotypes were evident. With one exception (OR-6635-20), the specimens from California and Oregon comprise distinct, reciprocally monophyletic clades that are only poorly differentiated from one another. Representative sequences for *P. odo-*

coilei, *P. tenuis*, and *P. andersoni* are deposited in GenBank as DQ371934–371951; all sequences for COX-II from *Parelaphostrongylus* spp. are reported here for the first time, with the exception of the two adults of *P. odocoilei* as noted.

Haplotype variation was also evident among other incomplete sequences derived from the larvae of two additional deer from Oregon (8408 and 8244) (Table 2). Overall, 16 of the 21 variable nucleotides represent synonymous third codon position substitutions. Thus, selective neutrality would seem to characterize most if not all of the evident population variation in this locus.

DISCUSSION

We provide the first unequivocal identification of *P. odocoilei* in Columbian black-tailed deer from Oregon. Previously, the supporting evidence for the occurrence of *P. odocoilei* in this population

TABLE 2. Variable nucleotides in cytochrome oxidase II (COX-II) among dorsal spine larvae from Oregon (OR) and California (CA) and adult *Parelaphostrongylus odocoilei* from the Mackenzie Mountains, Canada.^a

	Nucleotide position																				
	2	6	8	8	8	9	0	0	1	2	3	4	4	4	5	8	9	1	3	3	7
3rd codon positions	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Adult_USNPC_94329_DQ297381	.	A	G	T	A	T	A	G	C	A	T	A	T	G	T	G	A	C	T	A	.
Adult_USNPC_94333_DQ297382	.	A	G	T	A	T	A	G	C	A	T	A	T	G	T	G	A	C	T	A	.
CA_0105_1	T	A	A	T	A	C	G	G	T	G	A	A	C	T	T	G	T	C	T	G	C
CA_0105_2	T	A	A	T	A	C	G	G	T	G	G	A	C	T	T	A	T	C	T	A	.
CA_0105_3	T	G	A	T	A	C	G	G	T	G	G	A	C	T	T	G	T	C	T	A	.
CA_0113_1	T	A	A	T	A	T	G	G	T	G	G	A	C	T	T	G	T	C	T	A	C
CA_0127_1	T	A	A	T	A	T	G	G	T	G	G	A	C	T	T	G	T	C	T	A	T
CA_0127_2	T	A	A	T	A	C	G	G	T	G	G	A	C	T	C	G	T	C	T	A	C
OR_6635_1	C	A	A	C	G	C	G	A	C	G	G	A	C	G	T	G	T	C	T	A	.
OR_6635_2	.	A	A	T	A	C	G	G	T	G	G	A	C	T	C	G	T	C	.	.	.
OR_6635_3	.	A	A	C	A	C	G	G	C	G	G	A	C	T	T	G	T	T	.	.	.
OR_6684_1	.	A	A	C	G	C	G	G	C	G	G	A	C	T	T	G	T	C	.	.	.
OR_6684_2	C	A	A	C	G	C	G	A	C	G	G	A	C	T	T	G	T	C	T	A	T
OR_6684_3	.	A	A	C	G	C	G	G	C	G	G	A	C	T	T	G	T	T	.	.	.
OR_8244_1	.	.	A	C	G	C	G	G	C	G	G	A	T	T	T	G	T	C	A	.	.
OR_8244_2	C	G	G	C	G	G	A	C	T	T	G	T	C	A	.	.
OR_8408_1	G	G	C	T	T	G	T	C	.	.	.
OR_8408_2	G	A	C	T	T	G	T	C	A	.	.

^a 16/21 variable positions occur at the third codon position; missing data indicated by a period.

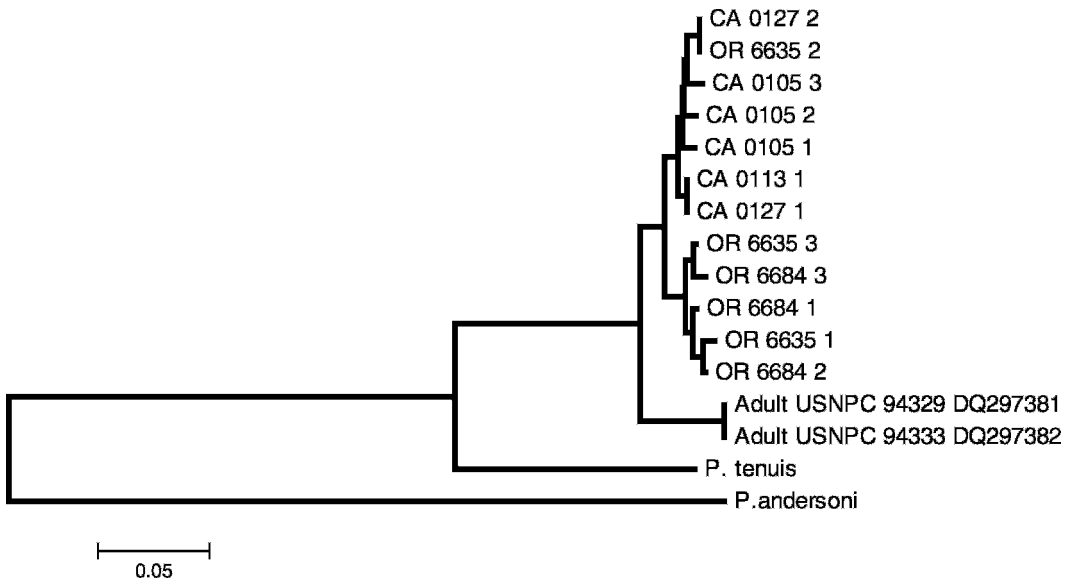


FIGURE 1. Midpoint-rooted Neighbor-Joining tree reconstructed from variation in a portion of the mitochondrial cytochrome oxidase II gene of dorsal-spined larvae (DSLs) collected from Columbia black-tailed deer in Oregon (OR) and California (CA). Equivalent topologies result from trees reconstructed using complete and pairwise deletion of gaps. Reconstruction reveals consistent separation (reciprocal monophyly) of *P. odocoilei* and the congeners *P. andersoni* and *P. tenuis*. This result and comparisons of larval sequences to those from validated adult specimens of *P. odocoilei* establishes the identity of DSLs from Oregon.

consisted mainly of the observation of DSLs in deer fecal samples collected in the northwestern region of Oregon during 1999 (Foreyt, 1999) and DSLs and eggs found in lung tissue of deer clinically affected with hair-loss syndrome (Bildfell et al., 2004; Foreyt et al., 2004). The overall geographic distribution, prevalence, and pathology of *P. odocoilei* remain to be determined in Oregon deer populations.

Prior knowledge had suggested that *P. odocoilei* occurred in a series of disjunct geographic foci across western North America involving Columbian black-tailed deer, mule deer, and mountain goats as definitive hosts (Lankester, 2001). The first records from Dall's sheep, from the Mackenzie Mountains, NT, Canada, indicated a considerably broader distribution for this nematode extending into the subarctic (Kutz et al., 2001). Evidence for an uninterrupted distribution for *P. odocoilei* in cervid and caprine hosts, from California to Alaska and the NT, has now

been clearly demonstrated (Jenkins et al., 2005).

Interestingly, with only one exception, the larvae sampled from Oregon and California seem genetically discrete, and both are partitioned from populations in the subarctic based on our preliminary comparisons (Fig. 1). If generally true, this partitioning would indicate that contact among their respective host populations is currently insufficient to promote substantial parasite gene flow. The diversity of COX-II haplotypes, and their apparent geographic differentiation, offer the promise that this locus may provide a basis for more extensively exploring their phylogeography and host associations, and a way to more easily discriminate among closely related species (Criscione et al., 2005; Hoberg, Abrams, Jenkins, Rosenthal, unpubl.).

Our study, however, does not rule out the possibility that other related species of *Parelaphostrongylus* may occur in northwestern North America. For example,

demonstrating *P. andersoni* in coastal Oregon would radically alter our current understanding of the geographic distribution of this parasite, because it has never been observed west of the Rocky Mountains. Although we do not have sufficient data to understand population genetic variation in *P. andersoni*, based on COX-II sequences it is clearly differentiated from *P. odocoilei* (reciprocal monophyly as established in Figure 1). *Parelaphostrongylus andersoni* has been reported in white-tailed deer at disjunct localities from the southeastern United States, northeastern Wyoming, and south central British Columbia, and it is typical in Barren-ground and woodland caribou at boreal to Arctic latitudes across the Nearctic (Lankester, 2001). There have been no substantiated records in *O. hemionus* from regions west of the Rocky Mountains, nor the far western Nearctic (Lankester, 2001). *Parelaphostrongylus odocoilei* and *P. andersoni* would be expected in sympatry at a minimum along the Cordillera from southern British Columbia to southern Alaska, and the Mackenzie Mountains of the NT and Yukon (Kutz et al., 2001; Lankester, 2001; Jenkins et al., 2005). The latter parasite, however, was not demonstrated in mule deer, Dall's sheep, or mountain goats during surveys for *P. odocoilei* across this region (Jenkins et al., 2005) or in phylogeographic studies now in progress (Hoberg, Abrams, Jenkins, Rosenthal, unpubl.).

Interestingly, before the discovery of a substantially more extensive geographic distribution of *P. odocoilei* (Kutz et al., 2001; Jenkins et al., 2005), there had been only six records for this elaphostrongyline in North America (Lankester, 2001). Clearly, this points to the need for both site-intensive and geospatially extensive sampling to reveal the limits of the geographic range and host association for species of *Parelaphostrongylus* in western North America. An area of specific interest should include eastern Oregon and Washington and the region of the Great

Basin extending across Idaho into Wyoming and Montana. Sampling protocols, consistent with the model developed by Jenkins et al. (2005), should include the following: 1) simultaneous collection of adult nematodes and DSLs from individual hosts, 2) identification of adult male worms based on comparative morphology, and 3) validating sequences for known adults and larvae. Validation of sequences, or the eventual development and application of PCR-based diagnostic markers, would provide the foundation for rapid and geographically extensive sampling of first-stage larvae derived solely from collection and extraction of feces and could serve in studies of population genetics, phylogeography, and molecular epidemiology (Hoberg et al., 2001; Criscione et al., 2005; Jenkins et al., 2005).

Sequencing a mitochondrial locus from adult and larval *P. odocoilei* and from *P. andersoni* and *P. tenuis* has established a basis to explore aspects of lungworm population genetics that would be difficult or impossible using ITS-2 alone. Our data confirm previous findings that little geographic differentiation occurs in the ITS-2 of broadly distributed specimens (Jenkins et al., 2005); by contrast, the worm populations of physically proximate sampling locales may differ by modest amounts of variation in the mitochondrial genome, as suggested by this preliminary study. Furthermore, our comparisons indicate that this mitochondrial locus seems to recover stronger phylogenetic signal differentiating the various species belonging to the genus than does the ITS-2, which may reflect the comparatively rapid evolutionary rate and nonrecombinant, uniparental inheritance of metazoan mitochondrial genomes.

Moreover, our interspecific comparisons suggest that sequence variation in this or other mitochondrial genes may provide greater means to resolve differences among such closely related taxa and to reconstruct their phylogeographic history. This increased resolution may be

attributed to elevated replacement rates of silent substitutions in mitochondrial genes, which are inherited without recombination solely through maternal lineages. Additionally, the interpretation of COX-II variation will not be complicated by the presence of intraindividual variation among duplicated gene copies, as is evident for the ITS-2 (Jenkins et al., 2005).

Documenting the host and geographic distribution of parasitic helminths in both wild and domestic ungulates is a matter of practical significance, because of the potential for host-switching at the interface of natural and managed ecosystems, and the possibility of translocation and introduction of parasites into new geographic regions or exposure of naïve hosts populations concomitant with the movement of deer and potentially caprines. Additionally, ongoing processes linked to anthropogenic changes in habitat and the expected ecological perturbation attendant to global climate change can be predicted to dramatically influence how pathogens and diseases are distributed in space and time (Hoberg, 1997; Daszak et al., 2000; Harvell et al., 2002; Hoberg et al., 2002; Kutz et al., 2004, 2005). Baselines established through faunal surveys and inventories remain necessary to define and understand such ecological shifts, and the consequences of emergent parasites and pathogens.

ACKNOWLEDGMENTS

We gratefully acknowledge Robert M. Timm and Robert J. Keiffer (University of California Hopland Research and Extension Center), Doug Cottam and Tami Wagner (Oregon Department of Fish and Wildlife), and Bob Sonn (Oregon State University Veterinary Diagnostic Laboratory) for invaluable assistance in obtaining samples from California and Oregon. Ramon Carreno and Steve Nadler (University of California, Davis) kindly provided specimens of *P. odocoilei* from research on protostrongylids and parasites in black-tailed deer. Furthermore, we thank Emily Jenkins, Susan Kutz, and Lydden Polley (Western College of Veterinary Medicine, University of Saskatchewan) for access to

comparative materials, specimens of *P. tenuis* and *P. andersoni* for sequencing, and valuable discussions on the broader geographic range for *P. odocoilei*. This project was funded in part by the Oregon Department of Fish and Wildlife.

LITERATURE CITED

- BENDER, L. C., AND P. B. HALL. 2004. Winter fawn survival in black-tailed deer populations affected by hair-loss syndrome. *Journal of Wildlife Diseases* 40: 444–451.
- BILDFELL, R. J., J. W. MERTINS, J. A. MORTENSON, AND D. F. COTTAM. 2004. Hair-loss syndrome in black-tailed deer of the Pacific Northwest. *Journal of Wildlife Diseases* 40: 670–681.
- CARRENO, R. A., AND M. W. LANKESTER. 1993. Additional information on the morphology of the Elaphostrongylinae (Nematoda: Protostrongylidae) of North America Cervidae. *Canadian Journal of Zoology* 71: 592–600.
- CRISCIONE, C. D., R. POULIN, AND M. S. BLOUIN. 2005. Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Molecular Ecology* 14: 2247–2257.
- DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2000. Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science* 287: 443–449.
- FOREYT, W. J. 1997. *Veterinary parasitology reference manual*, 4th Edition. Washington State University, Pullman, Washington, 190 pp.
- . 1999. Washington Animal Disease Diagnostic Laboratory Report, Pullman, Washington.
- , P. B. HALL, AND L. C. HALL. 2004. Evaluation of ivermectin for the treatment of hair-loss syndrome in black-tailed deer. *Journal of Wildlife Diseases* 40: 434–443.
- GAJADHAR, A., T. STEEVES-GURNSEY, J. KENDALL, M. LANKESTER, AND M. STEEN. 2000. Differentiation of dorsal-spined elaphostrongylinae larvae by polymerase chain reaction amplification of ITS-2 of rDNA. *Journal of Wildlife Diseases* 36: 713–722.
- GASSER, R. B., N. B. CHILTON, H. HOSTE, AND I. BEVERIDGE. 1993. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Research* 21: 2525–2526.
- , AND J. R. MONTI. 1997. Identification of parasitic nematodes by PCR-SSCP of ITS-2. *Molecular and Cellular Probes* 11: 201–209.
- GRAY, J. B., AND W. M. SAMUEL. 1986. *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) and a protostrongylid nematode in woodland caribou (*Rangifer tarandus caribou*) of Alberta, Canada. *Journal of Wildlife Diseases* 22: 48–50.
- HARVELL, D., C. E. MITCHELL, J. R. WARD, S. ALTIZER,

- A. P. DOBSON, R. S. OTSFELD, AND M. D. SAMUEL. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158–2162.
- HOBERG, E. P. 1997. Parasite diversity and emerging pathogens. In *Global genetic resources: Access, ownership and intellectual property rights*, K. E. Hoagland and A. Y. Rossman (eds.). Association of Systematics Collections, Washington DC, pp. 71–83.
- , A. A. KOCAN, AND L. G. RICKARD. 2001. Gastrointestinal strongyles in wild ruminants. In *Parasitic diseases of wild mammals*, W. M. Samuel, M. Pybus and A. A. Kocan (eds.). Iowa State University Press, Ames, Iowa, pp. 193–227.
- , S. J. KUTZ, J. NAGY, E. JENKINS, B. ELKIN, M. BRANIGAN, AND D. COOLEY. 2002. *Protostrongylus stilesi* (Nematoda: Protostrongylidae): Ecological isolation and putative host-switching between Dall's sheep and muskoxen in a contact zone. *Comparative Parasitology* 69: 1–9.
- , E. J. JENKINS, B. ROSENTHAL, M. WONG, E. F. ERBE, S. J. KUTZ, AND L. POLLEY. 2005. Caudal polymorphism and cephalic morphology among first stage larvae of *Parelaphostrongylus odocoilei* (Protostrongylidae: Elaphostrongylinae) in Dall's sheep from the Mackenzie Mountains, Canada. *Journal of Parasitology* 91: 1318–1325.
- HOBMAIER, A., AND M. HOBMAIER. 1934. *Elaphostrongylus odocoilei* n.sp., a new lungworm in black-tailed deer (*Odocoileus columbianus*). Description and life history. *Proceedings of the Society for Experimental Biology and Medicine* 31: 509–514.
- JENKINS, E. J. 2005. Ecological investigation of a new host-parasite relationship: *Parelaphostrongylus odocoilei* in thinhorn sheep (*Ovis dalli*). PhD Dissertation, University of Saskatchewan, Saskatchewan, Canada, 225 pp.
- , G. D. APPELYARD, E. P. HOBERG, B. M. ROSENTHAL, S. J. KUTZ, A. M. VEITCH, H. M. SCHWANTJE, B. T. ELKIN, AND L. POLLEY. 2005. Geographic distribution of the muscle-dwelling nematode *Parelaphostrongylus odocoilei* in North America, using molecular identification of first stage larvae. *Journal of Parasitology* 91: 574–584.
- JUNNILA, A. 2002. Characterization of the second internal transcribed spacer (ITS2) rDNA region among the Elaphostrongylinae (Nematoda: Protostrongylidae). MS Thesis. Lakehead University, Thunder Bay, Ontario, Canada, 80 pp.
- KUTZ, S. J., A. M. VEITCH, E. P. HOBERG, B. T. ELKIN, E. J. JENKINS, AND L. POLLEY. 2001. New host and geographic records for two protostrongylids in Dall's sheep. *Journal of Wildlife Diseases* 37: 761–774.
- , E. P. HOBERG, J. NAGY, L. POLLEY, AND B. ELKIN. 2004. "Emerging" parasitic infections in Arctic ungulates. *Integrative and Comparative Biology* 44: 109–118.
- , ———, L. POLLEY, AND E. JENKINS. 2005. Global warming is changing the dynamics of Arctic host-parasite systems. *Proceedings of the Royal Society London B* 272: 2571–2576.
- LANKESTER, M. W. 2001. Extra-pulmonary lungworms of cervids. In *Parasitic diseases of wild mammals*, 2nd Edition, W. M. Samuel, M. J. Pybus and A. A. Kocan (eds.). Iowa State University Press, Ames, Iowa, pp. 228–278.
- LIU, H., AND BECKENBACH, A. T. 1992. Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Molecular Phylogenetics and Evolution* 1: 41–52.
- PLATT, T. R., AND W. M. SAMUEL. 1978. A re-description and neotype designation for *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea). *The Journal of Parasitology* 46: 330–338.
- PYBUS, M. J., W. J. FOREYT, AND W. M. SAMUEL. 1984. Natural infections of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in several hosts and locations. *Proceedings of the Helminthological Society of Washington* 51: 338–340.
- SAMUEL, W. M., T. R. PLATT, AND S. M. KNISPEN-KRAUSE. 1985. Gastropod intermediate hosts and transmission of *Parelaphostrongylus odocoilei*, a muscle-inhabiting nematode of mule deer, *Odocoileus h. hemionus*, in Jasper National Park, Alberta. *Canadian Journal of Zoology* 63: 928–932.
- SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU, AND P. FLOOK. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. *Annals of the Entomology Society of America* 87: 651–701.
- SWOFFORD, D. S. 2001. PAUP 4.0b.10 computer program for MacIntosh. Sinauer Associates, Sunderland, Massachusetts.

Received for publication 12 June 2005.