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TRANSMISSION OF LUNGWORMS (*MUELLERIUS CAPILLARIS*) FROM DOMESTIC GOATS TO BIGHORN SHEEP ON COMMON PASTURE

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ABSTRACT: Four domestic goats (*Capra hircus*) that were passing first-stage dorsal-spined larvae of *Muellerius capillaris* were copastured on a 0.82-ha pasture for 11 mo from May 2003 to April 2004 with seven Rocky Mountain bighorn sheep (*Ovis canadensis*) that were not passing dorsal-spined larvae. During the 11-mo experiment, two bighorn sheep died from pneumonia caused by *Mannheimia (Pasteurella) haemolytica* biotype A, serotype 2. The remaining five bighorn sheep and the four domestic goats remained healthy throughout the experiment. *Muellerius* larvae were detected from all domestic goats on a monthly basis throughout the experiment and were first detected from all five surviving bighorn sheep approximately 5 mo after the copasturing began. Once the bighorn sheep began passing *Muellerius* larvae, larvae were detected in low numbers from all bighorn sheep every month thereafter for the 6 mo the goats were still in the enclosure and continued to pass larvae for more than 3 yr after the goats were removed from the experiment. Six bighorn sheep in two similar enclosures that did not contain goats did not pass *Muellerius* larvae before, during, or after the experimental period. Results of this experiment indicate that *M. capillaris* from domestic goats is capable of infecting bighorn sheep when animals are copastured together on a common range.

Key words: Bighorn sheep, experimental study, goats, lungworms, *Muellerius capillaris*, *Ovis canadensis*.

INTRODUCTION

Bighorn sheep (*Ovis canadensis*) throughout North America are commonly infected with lungworms of the genus *Protostrongylus*, which are relatively host-specific in wild sheep, and can be an important component in the multifactorial lungworm pneumonia complex (Forrester, 1971; Spraker et al., 1984). In many free-ranging bighorn sheep and thinhorn sheep (*Ovis dalli*) populations in North America, the prevalence of *Protostrongylus* spp. is 90–100% (Forrester, 1971; Uhazy et al., 1972; Pybus and Shave, 1984; Jenkins et al., 2005a; Foreyt, unpubl). Another lungworm, *Muellerius capillaris*, is a very common parasite of domestic goats and sheep and is an uncommon parasite in bighorn sheep in western North America. To our knowledge, it has only been reported from bighorn sheep in, or originating from, South Dakota (Pybus and Shave, 1984; Demartini and Davies, 1977). Demartini and Davies (1977) indi-

cated that *M. capillaris* was the major pathogen in an epizootic of pneumonia that occurred in 20 bighorn sheep that had been transported from South Dakota to Colorado. They suggested that *Muellerius* may have predisposed the bighorn sheep to bacterial pneumonia. In this experiment, we tested the hypothesis that *M. capillaris* from infected goats would infect bighorn sheep when copastured together. The rationale for the study was twofold. First, domestic goats are frequently used as pack animals and for weed control in bighorn sheep habitat. Second, lungworm larvae that were morphologically similar to *Muellerius* sp. were detected in a population of bighorn sheep in north-central Washington, USA, that experienced a pneumonia-related die off after sharing common habitat with a herd of domestic goats (Foreyt, pers. comm.). Because first-stage dorsal-spined larvae of *Muellerius* are morphologically similar to other lungworm larvae, such as *Parelaphostrongylus odocoilei* (Pybus and Shave, 1984), molec-

ular techniques, in addition to morphology, were used to identify the larvae.

MATERIALS AND METHODS

On 16 May 2003, four adult domestic goats that were passing dorsal-spined larvae morphologically identified as *M. capillaris* were purchased from two local goat producers and placed in a 0.82-ha outdoor pen at Washington State University (Pullman, Washington, USA), which contained seven resident Rocky Mountain bighorn sheep. The captive bighorn sheep (Table 1) had been in this pen for between 2 yr and 6 yr and were well acclimatized. The ground cover in the pen consisted of various grasses, numerous fallen dead pine trees, and large rocks. Based on fecal parasite evaluations done three times yearly for several years, dorsal-spined larvae had never been detected from these bighorn sheep or from any of the bighorn sheep that had lived in this pen for the past 20 yr. All bighorn sheep and goats were clinically healthy at the initiation of the experiment. All animals were copastured together for 11 mo; at which time, the goats were removed from the pen. Alfalfa (*Medicago sativa*) hay, an alfalfa-grain pelleted ration, and fresh water were available at all times. Animals were observed daily for clinical signs of illness.

For this experiment, fecal samples were collected from all bighorn sheep and goats at the time the goats were introduced into the pen and then collected monthly for 11 mo. Initially, goats were physically restrained and feces were removed manually from their rectums. The bighorn sheep were captured individually in a drive net, were physically restrained, and fecal samples were removed manually from their rectums. During subsequent months, fecal samples were collected monthly from all animals by following the animals in the pen and then collecting fecal samples from the ground after observing each animal defecate. Fecal samples were also collected at approximately 3-mo intervals from the surviving bighorn sheep for 3 yr after the goats were removed from the experiment. Fecal samples were evaluated for gastrointestinal parasites, using a standard sugar-flotation technique (sp gr=1.27), and for lungworm larvae, using a modified-Baermann technique (Foreyt, 2001). Dorsal-spined larvae from the domestic goats and bighorn sheep were preserved in 70% alcohol and were evaluated using the molecular techniques described by Gajadhar et al. (2000) and Jenkins et al. (2005a). Briefly, DNA was obtained from each larva by a brief incubation in larvae-extraction

TABLE 1. Numbers of *Muellerius* larvae recovered from bighorn sheep and domestic goat fecal samples.^a

Animal no.	Age (yr)	Sex	May	June	July	August	September	October	November	December	January	February	March
Bighorns													
Or11	3	M	0 ^b	0	12	36 ^b	18 ^b	13 ^b	15 ^b				
W27	2	F	0 ^b	0 ^b	0	0	0 ^b	6	31	74 ^b	2 ^b	5 ^b	50 ^b
W30	4	M	0	0 ^b	0 ^b	0 ^b	0 ^b	4 ^b	22	16 ^b	3 ^b	40 ^b	7
Y37	8	F	0 ^b	8 ^b	14 ^b	18 ^b	3 ^b	4 ^b	10 ^b				
R21	1 1/2	F	0	0 ^b	0	0 ^b	0	0	8	21	36	9	40
W33	lamb	M	0	0	0	dead							
W24	10	F	0 ^b	0 ^b	0 ^b	dead							
Goats													
W01	5	F	24	112	20	12	3	29	16	30	6	9	68
Y147	4	F	306	168	48	48	32	18	20	58	12	41	105
T1	2	F	2	3	10	14	2	11	29	17	42	16	5
S1	6	F	12	5	9	19	8	21	10	20	4	9	12

^a Numbers are expressed as numbers of larvae per gram of wet feces.

^b *Protostrongylus* sp. larvae detected.

buffer (proteinase K, polymerase chain reaction [PCR] buffer, and 2-mercaptoethanol). The samples were used immediately. The PCR was performed as described by Gajadhar et al. (2000), using the primers NC1 and NC2. Selected PCR products were cloned using the Topo TA cloning kit (Invitrogen, Burlington, Ontario, Canada) and sequenced in one direction (M13R) by the DNA Technologies Unit (National Research Council, Saskatoon, Saskatchewan, Canada). Multiple-DNA sequence-alignment analysis was performed using SeqMan and Megalign software (DNA Star, Madison, Wisconsin, USA), and alignment searches of the GenBank database. GenBank accession numbers for adult specimens from domestic sheep, identified as *Muellerius* based on morphology and subsequently sequenced as reference specimens, were AY679527 and AY679528.

Animals that died during the experiment were evaluated by the Washington Animal Disease Diagnostic Laboratory (WADDL, Pullman, Washington, USA), using standard necropsy and laboratory techniques. All air passages were examined grossly for parasites. Lung tissue samples for bacteriologic culture were inoculated onto blood and MacConkey agar plates for primary isolation. Isolates of *Mannheimia (Pasteurella) haemolytica* were biotyped according to sugar fermentation reactions (Biberstein, 1978) and were serotyped by results of a rapid agglutination test (Frank and Wessman, 1978).

RESULTS

Animal identification, age, and sex and numbers of *Muellerius* larvae recovered from all animals are listed in Table 1. Other gastrointestinal parasites recovered from fecal evaluations of both bighorn sheep and goats included low numbers of *Trichuris* sp., *Nematodirus* sp., *Eimeria* spp, and gastrointestinal strongyle nematodes. *Protostrongylus* sp. larvae were detected in samples from six of seven (86%) of the bighorn sheep during the experiment and were not detected in any of the goats (Table 1). All four goats (100%) and five (71%) of seven bighorn sheep remained healthy and survived the 11-mo copasturing experiment.

All goats continued to pass dorsal-spined larvae (evaluated monthly) throughout the 11 mo they were in contact with the

bighorn sheep (Table 1). Dorsal-spined larvae were not detected in fecal samples in any of the bighorn sheep at the initiation of the experiment. The five bighorn sheep that survived the experiment began passing dorsal-spined larvae approximately 5 mo after contact with the goats (Table 1) and continued to pass dorsal-spined larvae (evaluated monthly) for the next 5 mo and for the 3-yr period that fecal samples were collected following the removal of the goats. One bighorn lamb was born after the goats were removed from the experiment, and the lamb began passing dorsal-spined larvae when it was approximately 5 mo old.

A combination of comparative morphology and molecular analyses were used to identify the larvae. Initially, dorsal-spined larvae were identified as *Muellerius capillaris* for several reasons: 1) the morphology of the tail with the dorsal spine; 2) the small size of the larvae, which were approximately 350 μm in length (Pybus and Shave, 1984); and 3) *M. capillaris* is the lungworm commonly found in domestic goats. Larvae from both the bighorn sheep and goats were later verified as *M. capillaris* by comparing the sequence of the internal transcribed spacer (ITS-2) region of ribosomal DNA. GenBank accession numbers for the nucleotide sequences are AY679529 for the bighorn sheep isolate and AY679530 for the domestic goat isolate.

Two bighorn sheep died of bacterial pneumonia while the domestic goats were in contact with them. Bighorn W33, a 12-wk-old male that weighed 25.0 kg, and W24, a 10-yr-old ewe that weighed 57 kg died unexpectedly during the third month of the experiment. Approximately 75% of the lung parenchyma in each sheep was dark red and firm. All other organs appeared normal. Histopathologic lesions were similar in both sheep and indicated large areas of hemorrhage throughout approximately 90% of the alveolar spaces. Mats of bacteria were surrounded by distorted spindle cells and mature and degenerate neutrophils. The gross and histologic diagnosis was severe broncho-

pneumonia with intralesional bacteria. Large numbers of *Mannheimia haemolytica* biotype A, serotype 2, were isolated from the lungs.

DISCUSSION

Results from this experiment clearly indicated that *M. capillaris* from domestic goats can infect and mature in bighorn sheep that share common habitat with infected goats. All five of the surviving bighorn sheep passed *M. capillaris* larvae beginning 5–6 mo after copasturing with infected goats. All bighorn sheep continued to pass *Muellerius* larvae for more than 3 yr, indicating a persistence of infection or reinfection. Several species of gastropods are intermediate hosts for *M. capillaris* (Soulsby, 1965). Development time in the gastropod is 8 days at 25 C to 98 days at 5 C, and infective larvae apparently live in the gastropod for the 12–18 mo of the gastropod's life (Rose, 1957). During the experimental period, temperatures generally ranged between 4 C and 32 C. In addition to temperature, development time is dependant on species of gastropod intermediate host (Rose, 1957). The prepatent period in goats has been reported to be between 25 days and 38 days (Soulsby, 1965). The time for sufficient larvae from the infected goats to accumulate on pasture, develop in gastropods on pasture, and develop to maturity in the bighorn sheep supports the 5–6 mo delay in larval shedding by the bighorn sheep.

Results from this copasturing experiment also reinforce the concept of parasite and disease emergence in wildlife populations that can be associated with a change in climate, with movement of a new species of wildlife or domestic livestock into occupied native wildlife populations, or with increased population densities (Kutz et al., 2004). Of these primary factors, movement of animals and disease vectors are likely the most important causes of disease emergence (Daszak et

al., 2000; Deem et al., 2001; Kutz et al., 2004).

Although *Muellerius* is currently a rare finding in free-ranging bighorn sheep in western North America, it is now apparent that it can be cross-transmitted on range shared with infected domestic goats via a variety of gastropod intermediate hosts, and such infections are likely to become more prevalent with increased exposure to domestic goats on common ranges. In a theoretical risk assessment for wild sheep and goats, and domestic sheep and goats, *Muellerius* was classified as high risk for transmission (Garde et al., 2005).

Two bighorn sheep, including one that had been passing *Protostrongylus* sp. larvae died from *M. haemolytica* pneumonia during the early part of the experiment. *Muellerius capillaris* is a recognized pathogen in goats and is associated with widespread interstitial pneumonia (Nimmo, 1979; Kanwar et al., 1998). The association of *M. capillaris* and pneumonia in a goat has been reported (Young and Griffin, 1985), but the association of *M. capillaris* and pneumonia in these two bighorns could not be determined. It has been documented previously that *M. haemolytica* A2 from domestic sheep can be lethal in bighorn sheep (Foreyt et al., 1994), but the potential deleterious effects of *M. haemolytica* of goat origin in bighorn sheep has not been clearly documented. In an earlier report of a pneumonic epizootic in captive bighorn sheep in Colorado, USA, the entire herd of 20 sheep died, and all were infected with *Muellerius* (Demartini and Davies, 1977). Large numbers of adults, eggs, and larvae were detected in the lungs of all sheep at necropsy. The authors suggested that the presence of the parasites in the lungs may have predisposed the bighorns to pneumonia by obstructing airways, by disseminating bacteria, or by causing immune depression of the hosts. At the beginning of an all-age pneumonia outbreak in bighorn sheep in Hells Canyon in Washington, Idaho and Oregon, USA, identical

M. haemolytica organisms were isolated from a feral goat and two bighorn sheep that were in close contact (Rudolph et al., 2003). A large-scale pneumonia outbreak, in which over 300 bighorn sheep died followed the initial goat and bighorn association, further illustrates the potential for cross-transmission of pathogens between goats and bighorn sheep.

Adult *M. capillaris* are often within the alveoli and enclosed in nodules, which are 1 mm to several centimeters in diameter, but are also associated with diffuse lesions with irregular patches visible externally on lung tissue and with thickened alveolar walls. Lesions are generally composed of lymphocytes, ova, larvae, and adult worms (Sauerlander, 1988). In the current experiment, the five bighorns that passed *Muellerius* larvae during the experiment were not euthanized, and lesions could not be described. In goats, *M. capillaris* infections are often mild but can cause dyspnea and coughing and may predispose the animal to other infections because of mechanical damage primarily from migrating parasites (Nimmo, 1979). We did not detect developing lungworms grossly at necropsy or histologically in the limited number of lung sections examined from the two bighorns that died from pneumonia, but it is possible that *M. capillaris*, or *M. capillaris* in combination with *Protostrongylus*, may have been a predisposing factor in their deaths. Adult worms, larvae, and eggs of both species of lungworms accumulate in the lungs and can cause significant pulmonary pathology. Pneumonia epizootics in bighorn sheep and isolated cases of pneumonia in Dall's sheep (*Ovis dalli dalli*) have been linked to environmental stressors in combination with bacteria and lungworms (Bunch et al., 1999; Jenkins et al., 2000, 2007), but stress was not apparent in our experiment. At the time preceding the deaths of the two bighorns, the weather was mild, and goats and bighorns freely associated with each other, including bedding together.

Because of the close morphologic sim-

ilarity and polymorphism of first-stage larvae among several of the protostrongylid nematodes (Hoberg et al., 2005; Jenkins et al., 2005b), a combination of morphology and molecular techniques were used to positively identify the *Muellerius* larvae from both hosts. Use of molecular techniques in combination with morphology has become a reliable method for parasite identification and has been especially useful for evaluating host association and geographic distribution of parasite populations (Jenkins et al., 2005a).

Based on the results of this experimental study, bighorn sheep that occupy habitat with domestic goats are at potential risk of acquiring *Muellerius* infections, thus, increasing the potential risk of verminous pneumonia with possible concurrent or secondary bacterial pneumonia. Therefore, prudent management of bighorn sheep populations should minimize habitat sharing between the two species. Further work to evaluate the effects of *Muellerius* on bighorn sheep health is warranted.

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