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OCCURRENCE, DIAGNOSIS, AND STRAIN TYPING OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* INFECTION IN ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*) IN SOUTHWESTERN ALBERTA

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ABSTRACT: The role that wildlife may play in the transmission of *Mycobacterium avium* subspecies *paratuberculosis* (Map), the causative agent of Johne's disease (JD), and the potential consequences of infection in these populations are being given increasing consideration. A yearling male Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) from southwestern Alberta, Canada, was found infected with Map in August 2009. Clinical signs of emaciation and diarrhea and histologic findings of diffuse granulomatous enteritis of the distal ileum, lymphadenitis of the mesenteric lymph nodes, and lymphangitis of the ileum were similar to previously described cases of JD in bighorn sheep. Infection with Map was confirmed by bacterial isolation through fecal culture, acid-fast staining, and polymerase chain reaction (PCR) of IS*900*. The *Map1506* gene was sequenced, and the isolate was identified as a Cattle (Type II) strain. In a follow-up herd-level survey, three of 44 fecal samples (7%) from individual bighorn sheep from the same herd as the index case were PCR-positive and identified as Type II Map strains. Twenty-five samples from a distant bighorn population were negative. Additional strain typing of the isolates from the index case and the positive fecal samples was done by sequencing three discriminatory short sequence repeat (SSR) regions. All four SSR profiles differed from one another, suggesting multiple introductions or a long-existing circulation of Map within this bighorn population. Detailed molecular analyses are essential for understanding and managing diseases at the wildlife-livestock interface.

Key words: Bighorn sheep, epidemiology, Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis*, strain typing, wildlife-livestock interface.

INTRODUCTION

Johne's disease (JD), or paratuberculosis, is a chronic enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map), an intracellular, acid-fast bacterium characterized by slow growth in culture. Ruminants are the most common host for Map; however, it has been isolated from a wide variety of nonruminant species (Williams, 2001). Although most of the literature on Map focuses on production animal species where infection has significant economic implications, the potential role of wildlife in the epidemiology of paratuberculosis is of increasing interest because of the potential for interspecies transmission and the impact Map may have on the health of wildlife species (Motiwala et al., 2004).

Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) are native to the mountainous regions of western Canada and the United States. This species is ecologically fragile because of its particular habitat requirements, compounded by increasing habitat fragmentation (Valdez and Krausman, 1999). Infectious diseases, primarily in the form of bacterial pneumonias, have been an important limiting factor for many populations (Weiser et al., 2003). Clinical and subclinical infections with Map have been reported sporadically for bighorn sheep in Colorado and Wyoming, USA, and from captive bighorn, Dall's (*Ovis dalli dalli*), and Stone's sheep (*Ovis dalli stonei*) in Yukon, Canada (Williams et al., 1979, 1983a; Williams and Hibler, 1982; Garde et al., 2005).

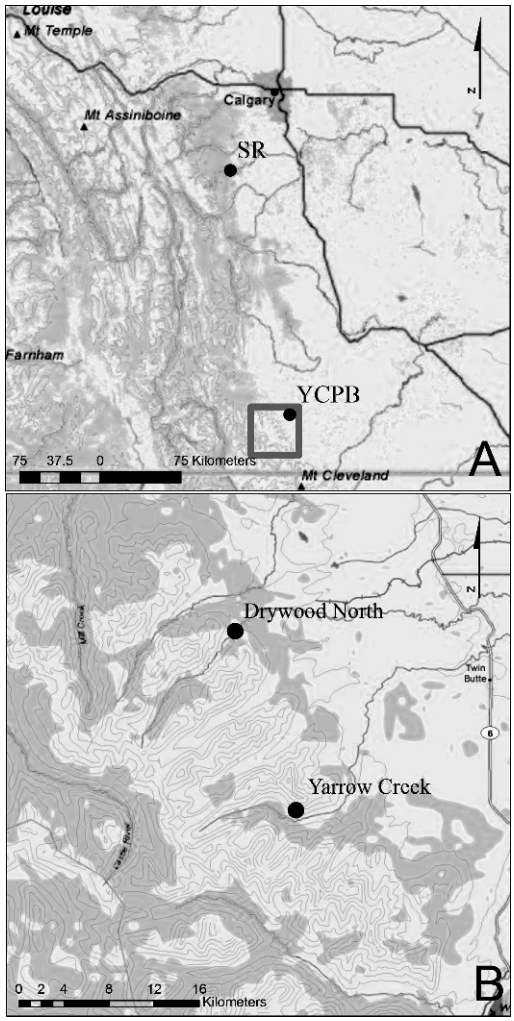

FIGURE 1. Study sites. (A) Location of the Yarrow Creek–Prairie Bluff (YCPB) and Sheep River (SR) bighorn sheep herds in southwestern Alberta, Canada. (B) Enlargement of the YCPB herd area with locations of bighorn sheep fecal samples found positive for *Mycobacterium avium* subspecies *paratuberculosis*.

However, there has been no systematic surveillance for Map in wild sheep of North America, and its role in population dynamics is unknown.

Approximately 11,000 Rocky Mountain bighorn sheep range in the high mountains of western Alberta, Canada, along the southern half of the Alberta-British Columbia border (Jorgenson, 2008). The bighorn sheep occur in small local bands that overlap nearby bands to loosely form a distinct herd, often determined by availability of suitable wintering areas. Although directed disease surveillance in bighorn sheep is limited, there is ongoing passive surveillance by a wide range of government and public observers. Bighorn sheep mortality events are known across the species range, and there is sensitized, passive surveillance for sick or dead bighorn sheep. All such reports and observations provided to the Alberta Fish and Wildlife Division are investigated. An outbreak of pneumonia in southwestern Alberta, Canada, in 1982–83 caused by *Pasteurella hemolytica* resulted in significant bighorn sheep mortality (Jokinen et al., 2008) and further sensitized local wildlife managers and wildlife enthusiasts. We report a case of JD in a bighorn sheep from the Yarrow Creek–Prairie Bluff bighorn sheep population in southwestern Alberta, Canada, and subsequent fecal surveys for Map in this and a second population approximately 200 km to the north. This survey was performed to ascertain the extent of the Map infection in these herds and to gather baseline data for future monitoring.

MATERIALS AND METHODS

Study sites

In Pincher Creek County in the far southwest corner of Alberta, Canada, the Yarrow Creek–Prairie Bluff (YCPB) bighorn sheep population ($n=179$; Fig. 1) overlaps with the Table-Castle band to the north ($n=29$) and the Barnaby Ridge band to the west ($n=45$; Alberta Fish and Wildlife, unpubl. data), as well as with multiple bighorn sheep bands in Waterton Lakes National Park (Alberta, Canada). Young rams in particular wander among the bands. Within their normal summer and winter ranges, bighorn sheep from the YCPB band are syntopic with a wide range of wild and domestic ungulates, including elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and moose (*Alces alces*), as well as domestic cattle (*Bos taurus*), sheep (*Ovis aries*), and feral horses (*Equus caballus*).

The Sheep River (SR) study site in Kananaskis Country (Fig. 1A) has been closely monitored since 1986 (Jorgenson et al., 1997;

Ruckstuhl et al., 2003). All animals in this population are ear-tagged, and detailed information regularly collected on individually identified bighorn sheep includes group interactions, dominance ranks, mating behavior, and any apparent health issues. The permanent population is composed of about 40 individuals, with 30–40 other bighorn sheep that reside temporarily. There is extensive cattle ranching throughout this area, consisting primarily of cow-calf beef cattle operations, providing occasions for interaction between wildlife and domestic species (Statistics Canada, 2008). Although individuals from the YCPB and SR populations are unlikely to come into direct contact, bighorn sheep occur throughout the mountainous regions between these populations.

Clinical case

In late August 2009, oil workers in the area of the YCPB herd reported a yearling bighorn ram that was lethargic and in poor body condition. The animal was located by Alberta Fish and Wildlife Staff (49°11′37.03″N, 114°0′05.45″W) at 1,656 m elevation. It was recumbent and allowed staff to approach closely; no coughing or nasal discharge was observed. The ram was humanely euthanized on 25 August, stored in a cooler on ice overnight, and transported to the University of Calgary, Faculty of Veterinary Medicine the following day.

Complete necropsy and histopathologic examinations were performed. Sections of the ileum and mesenteric lymph nodes were fixed in 10% neutral-buffered formalin, sectioned, and stained with hematoxylin and eosin. Additional sections of the ileum and mesenteric lymph node were stained with the acid-fast Ziehl-Neelsen stain. Feces were collected from the rectum during necropsy and kept frozen at −20 C until processed for culture.

Herd-level survey

The necessary sample size for within-herd disease detection at the 5% prevalence level (80% power, 95% confidence level) was calculated using WinEpiscope 2.0. These were calculated to be 46 and 31 animals for the YCPB and SR bighorn sheep populations, respectively. Fecal samples were collected between November 2009 and June 2010 from individually identified bighorn sheep at the two study sites by observing animals from a distance and retrieving pellets within 5–15 min. of defecation. Information collected for each sample included the sex and approximate age of the animal and the location, date, and time of collection. Each sample was collected with new disposable gloves, stored in Ziploc bags, and transported on ice.

Laboratory diagnostics

Fecal decontamination was performed on all fecal samples collected using the ESP para-JEM specimen decontamination protocol (TREK Diagnostic Systems, Cleveland, Ohio, USA). Samples were prepared for liquid culture using ESP para-JEM culture solution and incubated at 37 C in the ESP Culture System II Trek Machine for 7 wk. For the clinical case, approximately 100 µl of the decontaminated inoculate was aliquoted onto a Herrold's egg yolk (HEY) slant as an additional confirmatory step.

A genomic DNA extraction protocol was performed on all liquid culture broths following incubation: Trek bottles were vortexed before removing 200 µl of the culture, which was added to 800 µl of 95–100% ethanol in a 1.5-ml Eppendorf tube. This mixture was centrifuged for 10 min at 4,200 × G. The supernatant was discarded before performing a wash step: the pellet was resuspended in 900 µl of sterile Gibco Dulbecco's phosphate-buffered saline (DPBS; Invitrogen, Grand Island, New York, USA) and centrifuged for 10 min at 4,200 × G. This wash step with centrifugation was repeated after discarding the supernatant. After again discarding the supernatant, the pellet was resuspended in 100 µl of sterile Gibco DNase/RNase- free water (Invitrogen). This mixture was boiled for 25–30 min and centrifuged at 4,800 × G for 2.5 min. The extracted DNA was removed into a new Eppendorf tube and the pellet discarded.

DNA was also extracted from bacterial colonies on the HEY slant from the clinical case after 40 days and used in PCR: 20 µl of DPBS, 180 µl of ATL tissue lysis buffer (Qiagen, Mississauga, Ontario, Canada) and colonies from the HEY slant were bead-beated in conical tubes with 0.4 g of 0.1 mm silica beads for 2 min. The Spin-Column protocol from the DNeasy blood and tissue kit (Qiagen) was then followed beginning at step two.

We performed PCR targeting IS*900* as previously described (Vary et al., 1990) with some modifications: 5 µl of lysate was added to the reaction mixture as described, containing 1.25 U Top Taq (Top Taq PCR kit; Qiagen), creating a reaction volume of 50 µl. Strain typing was performed on IS*900* positive samples by amplifying the 5′ end of *Map1506* locus as previously described (Castellanos et al., 2010). For the clinical case, the PCR product was purified using the QIAquick PCR

TABLE 1. Real-time polymerase chain reaction (RT-PCR) conditions used for the amplification of three short sequence repeat (SSR) regions (G1, G2, and GGT) of *Mycobacterium avium* subspecies *paratuberculosis* isolated from Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in southwestern Alberta, Canada, 2009–2010.

SSR locus	Nucleotide position[a]	Forward primer sequence	Reverse primer sequence	RT-PCR reaction conditions
G1	1793010	5′-GTTGTGCCGGG-CGGCGTTTG-3′	5′-GGTCCGCGC-CAACGGGTG-3′	95 C for 3 min; 34 cycles at 95 C for 10 sec, and annealing/ extension at 70 C for 20 sec
G2	2719017	5′-GTAGTCCGCAT-CCCGTGCCG-3′	5′-CGATGCCCAT-GAGCGTGCCG-3′	95 C for 3 min; 36 cycles at 95 C for 10 sec, and annealing/ extension at 68 C for 20 sec
GGT	1028061	5′-TGCACAACCGG-GTCAGCTCG-3′	5′-AACCCCTGCAC-GGTGCGTTC-3′	95 C for 3 min; 36 cycles at 95 C for 10 sec, and annealing/ extension at 68 C for 20 sec

[a] Position of forward primer in relation to K10 *Mycobacterium avium* subspecies *paratuberculosis* reference strain, NCBI Reference Sequence NC_002944.2.

Purification Kit (Qiagen). For the three other cases, PCR products were extracted from a 1% agarose gel using the QIAquick gel extraction kit (Qiagen) to isolate the desired amplicon from nonspecific DNA amplification. Products were sequenced using forward and reverse primers (Castellanos et al., 2010; University Core DNA Services, University of Calgary, Alberta, Canada), and aligned with the Map1506 gene locus sequence of the K10 strain (National Center for Biotechnology Information [NCBI] Ref: NC_002944.2) using Geneious Pro 5.0.4 (Drummond et al., 2010). Sequencing of three Short Sequence Repeat (SSR) regions (G1, G2, and GGT) that have been previously described for the characterization of Map strains based on the presence of polymorphisms (El-Sayed et al., 2009) was also performed for further strain discrimination, using the conditions described in Table 1. Primers were designed using NCBI primer-BLAST. All PCR amplification for sequencing was performed with real-time PCR using SsoFast EvaGreen supermix (Bio-Rad, Mississauga, Ontario, Canada) on a Bio-Rad CFX96 thermocycler. To confirm sequencing results of SSR regions G1 and G2, a second PCR run was performed with the same primers using the Phusion High Fidelity PCR kit (New England Biolabs, Pickering, Ontario, Canada).

RESULTS

Clinical case

On postmortem examination, the ram was in poor body condition with diffuse muscle atrophy around the spine, neck, and rear quarters and minimal subcutaneous and visceral fat stores. A large amount of dried fecal matter was present around the perineum and on the hind legs (consistent with diarrhea). Scant formed feces were present within the rectum. Approximately 0.75 m of the distal ileum had mild to moderate thickening of the submucosa with increased prominence of mucosal rugae. The mesenteric lymph nodes were mildly enlarged. Grass, dirt, and small stones were present in the rumen, reticulum, and omasum.

On histologic examination of the ileum and mesenteric lymph nodes, there was a severe, diffuse, lepromatous granulomatous enteritis; lymphadenitis; and lymphangitis. In the ileum, there was diffuse moderate to marked infiltration and expansion of the lamina propria of the mucosa, the submucosa, and effacement of Peyer's patches with sheets of epithelioid macrophages and lesser numbers of small mature lymphocytes and multinucleated giant cells including Langhans-type giant cells. Epithelioid macrophages and multinucleated giant cells had a fine granularity to their cytoplasm (intracytoplasmic bacilli). Multifocal small areas of lytic necrosis characterized by cytoplasmic and nuclear karyorrhectic debris were present within the sheets of macrophages. Serosal lymphatics were surrounded by

TABLE 2. Strain-typing results for four isolates of *Mycobacterium avium* subspecies *paratuberculosis* (Map) from the Yarrow Creek–Prairie Bluff bighorn sheep herd (*Ovis Canadensis*) in southwestern Alberta, Canada. The clinical case was sampled in August 2009; the remaining three isolates were from fecal samples collected in June 2010.

					SSR[a,b]		
Bighorn sheep ID no.	Location	Age	Sex	Map1506	G1	G2	GGT
Clinical case							
UC130	Yarrow Creek	Yearling	Male	Type II	7	12	4
Herd-level survey							
BS10-39	Drywood North	2–3 yr	Male	Type II	10	10	5
BS10-58	Yarrow Creek	Adult	Female	Type II	7	11	4
BS10-77	Yarrow Creek	Adult	Female	Type II	7	10	4

[a] SSR = short sequence repeat; ID = identification.

[b] Number of repeated units are reported for each of three loci.

aggregates of epithelioid macrophages and small mature lymphocytes and luminally contained fibrin thrombi. In the mesenteric lymph node, approximately 70% of the cortex and paracortex was effaced and expanded by sheets of epithelioid macrophages and lesser numbers of multinucleated Langhans-type giant cells, similar to those described in the intestine. There was moderate expansion of the medullary sinuses with epithelioid macrophages. Multifocal areas of coagulative necrosis were present in the cortex, as well as occasional reactive germinal centers. Three large, afferent lymphatics in the perinodal fat contained large luminal fibrin thrombi with moderate infiltrations of lymphocytes and macrophages in the adjacent adipose tissue. A focal area of mineralization was noted in the perinodal fat.

Enteritis and lymphadenitis were graded based on published grading schemes for JD in domestic sheep (Dennis et al., 2011). The number of epithelioid macrophages was graded as 4/4 (confluent sheets that distort architecture), terminal ileal lesions grade as 2/3 (epithelioid macrophages within the lamina propria and submucosal or heavy involvement of interfollicular areas of Peyer patches) and mesenteric lymph nodes grade a grade 3/3 (epithelioid macrophages expand subcapsular or medullary sinuses or are within/around capsular lymphatic vessels), producing a cumulative grade of 9/10, consistent with severe disease.

Ziehl-Neelsen–stained sections of the ileum and lymph node demonstrated that diffusely, most macrophages in areas of granulomatous inflammation were distended with >10 acid fast bacteria, consistent with a grade 4/4 in the AFB grading scheme for JD in domestic sheep (Dennis et al., 2011). This is consistent with the multibacillary form of JD.

Viable Map was cultured from both the Trek-ESP liquid culture and from the HEY slant, with growth first detected on day 12 and 20 respectively. Sequencing of the *Map1506* gene showed that it was identical to the K10 reference strain in Genbank, which is a known Type II (Cattle) strain. This gene has been previously shown to be discriminatory between strain types I, II and III based on single nucleotide polymorphisms at a minimum of five different base pairs (Griffiths et al., 2008). Sequencing of the three SSR regions revealed the strain to have 7G1, 12G2, and 4GGT repeats (Table 2).

Herd-level survey

Fecal samples from 44 and 25 individual bighorn sheep were collected from the YCPB and SR herds, respectively. All fecal samples from both sites were culture negative. However, three samples (7%) from the YCPB herd were positive by

PCR of the IS*900* region, whereas none of the samples obtained from the SR herd were positive by PCR. All three PCR-positive samples from the YCPB herd were Type II strains, but sequencing of the three SSR regions resulted in three different genotypes from that of the clinical case (Table 2). Two of the PCR-positive samples from the YCPB herd were collected at a site close to the index case (Yarrow Creek), whereas the third originated from a separate river valley (Drywood North), approximately 15 km away (Fig. 1B). Sequencing results of G1 and G2 loci were confirmed by a second round of sequencing from the additional PCR run using the Phusion enzyme.

DISCUSSION

Necropsy, histopathology, culture, and PCR results confirmed Map Type II (Cattle strain) to be the causative agent of the clinical signs found in this bighorn ram. The clinical presentation, gross and histopathologic findings were similar to previous cases of JD in bighorn sheep (Williams et al., 1983a), although intestinal thickening was more marked than what has been described. Microscopic lesions of the gastrointestinal tract were limited to the ileum, whereas previous cases had lesions ranging from the proximal jejunum to the proximal colon, often involving the cecum (Williams et al., 1983a). A range of histologic lesions occurs in cases of JD in domestic sheep, which seems to follow a progression associated with changes in immune response (Pérez et al., 1996; Balseiro et al., 2008). The diffuse lesions described in this case and numbers of acid-fast staining bacteria within macrophages correspond most closely to those of the multibacillary form (Clarke and Little, 1996; González et al., 2005; Balseiro et al., 2008). This form of JD is thought to be the result of a low cellular immune response and high humoral response, consistent with a late stage of the disease (Clarke, 1997). Bighorn sheep may present with clinical signs of Map infection as early as 1 yr of age, whereas domestic sheep do not generally show clinical signs until 2–3 yr of age (Williams et al., 1983a; Begg and Whittington, 2010). Although there are insufficient reports of JD in bighorn sheep to determine the average age of onset of clinical disease, it would be valuable to investigate whether there are differences in the host immune response of this species that allow for early onset of disease.

Strain typing of Map is critical for understanding transmission pathways and differences in host preference. *Mycobacterium avium* subspecies *paratuberculosis* has been classified into two broad, genetically distinct strains that differ in their growth characteristics, host range, and pathogenicity: Type I (sheep or "S" type) and Type II (cattle or "C" type; Stevenson, 2010). A third Intermediate type (Type III) has been described, although it is considered to be closely related to Type I (Möbius et al., 2009). Some of the techniques that have been described for distinguishing between these major strain types include restriction fragment length polymorphism (RFLP) analysis of IS*900*, PCR-restriction endonuclease analysis of IS*1311*, PCR of a discriminatory region, and sequencing or high resolution melt analysis of Map1506 (Marsh et al., 1999; Whittington et al., 2000; Collins et al., 2002; Griffiths et al., 2008; Castellanos et al., 2009). Domestic sheep and cattle are most commonly infected with their named strain type respectively, suggesting that there may be a certain degree of host specificity (Whittington et al., 2000). However, neither strain is entirely specific for a single ruminant species, but both are infective for a variety of hosts, both naturally and experimentally (Ris et al., 1987; Pavlík et al., 1995; Stewart et al., 2004). Type I strains have rarely been isolated from wildlife species (Stevenson, 2010). As expected, all four isolates from this bighorn sheep herd were Type II strains; in the limited literature in which strain typing has

been performed on North American Map isolates, we were unable to find reports of Type I strains in either wildlife or livestock on this continent. A Type III strain has been reported once in domestic sheep in Canada (Collins et al., 1990).

Mycobacterium avium subspecies *paratuberculosis* has a restricted level of genetic diversity, and polymorphic SSRs (microsatellite regions) provide one of the stronger tools for discriminating between distinct isolates (Amonsin et al., 2004; El-Sayed et al., 2009). These particular regions within the genome have a higher level of variability because of errors in DNA replication through recombination or slipped-strand mispairing (Motiwala et al., 2004). Multilocus SSR (MLSSR) gives a high discrimination index (Douarre et al., 2011), may group Map isolates into meaningful clusters based on host species and geographic origin (Amonsin et al., 2004), and provides strong evidence to support the transmission of identical strains within herds (Motiwala et al., 2005; Harris et al., 2006; Pradhan et al., 2011). For these reasons, MLSSR is thought to be a more useful tool for molecular epidemiologic investigation than commonly used typing methods such as IS*900* RFLP (Collins, 2010). Additionally, the use of PCR-based SSR typing enabled us to apply the same typing method to all four of our isolates, including the culture-negative samples.

Three of the bighorn sheep typed in this study, including the index case, had the 7G1-4GGT type, the most common profile in previous studies (Motiwala et al., 2005; El-Sayed et al., 2009). Although all four positive samples were obtained from the YCPB herd, the three samples with this particular profile were clustered in the southern area of this herd's range, whereas the fourth sample with a 10G1-5GGT profile came from an individual at the northwestern edge of this herd's range. If the third SSR locus is also considered, all four Map isolates had distinct genotypes. Thus, a previous finding that isolates from the same wildlife species tend to cluster by allele type (Motiwala et al., 2004) was not supported by this study.

A high amount of variation in SSR profiles from Map isolates from dairy cows within a restricted geographic area has been found (El-Sayed et al., 2009). Other investigators found multiple genotypes in the same region or on the same premises (Motiwala et al., 2005; Harris et al., 2006; van Hulzen et al., 2011), and cases were reported wherein more than one Map genotype was detected in an animal (El-Sayed et al., 2009; Pradhan et al., 2011).

The interpretation of the presence of different genotypes within the same herd is challenging. The presence of multiple strains within the same facility or farm could be explained by multiple introduction events (Motiwala et al., 2004), and the diversity of Map isolates has been positively associated with the rate of introduction of new animals to livestock herds (Möbius et al., 2008). In our case, the presence of multiple strains could be due to contact with cattle carrying different strain types. Conversely, if Map were circulating within the bighorn sheep population, multiple strain types could arise over time due to natural mutations. Further investigation into the mechanisms of allele mutation of SSR regions and strain transmission is warranted to have stronger grounds for the interpretation of MLSSR Map typing results. Additionally, strain typing of Map isolates from livestock in the area would allow interspecies comparison and likely provide insight on transmission patterns at the wildlife-livestock interface.

Investigators performing sequencing of loci containing repetitive DNA have noted challenges because of amplification errors during PCR. A high error rate in DNA replication by PCR was documented for regions with high mononucleotide and dinucleotide repeats (Clarke et al., 2001). Their observation that monomeric repeats of more than 11 base pairs were replicated with lower accuracy was confirmed by

other investigators (Thibault et al., 2008). Fazekas et al. (2010) found that the accuracy for amplification of repeated mononucleotide sequences could be improved up to a length of 15 base pairs with fusion polymerases. Fusion polymerases are proofreading DNA polymerases fused to a nonspecific, double-stranded DNA binding domain, resulting in a larger area of contact between the enzyme and the double-stranded DNA that is being replicated. The authors hypothesized that DNA polymerases have a tendency to dissociate from the DNA when their active site is filled by a repetitive sequence, an issue that is partially countered by the increased contact surface of fusion enzymes. Although the SsoFast EvaGreen supermix uses a fusion DNA polymerase, we chose to perform a confirmatory PCR run using the Phusion enzyme that is reported to give highly accurate results in the amplification of GC-rich sequences (Shi and Jarvis, 2006). Identical results were produced in both PCR runs, demonstrating reproducibility of amplification and sequencing results with these enzymes.

In conclusion, the occurrence of JD in a wild bighorn sheep in southwestern Alberta, Canada, and the subsequent detection of Map in feces of individuals from within the same herd has potential management implications for wildlife and livestock. The detection of an additional three Map-positive animals in the YCPB bighorn sheep herd based on fecal PCR is a significant finding. In other species, it is known that the sensitivity of fecal culture and PCR is not as high as it is for tissue samples (Whittington and Sergeant, 2001; Huntley et al., 2005). Consequently, the number of detected cases is likely an underestimation of the true level of infection in this bighorn sheep population. This report reconfirms the susceptibility of this species to JD and highlights the importance of monitoring for this pathogen in bighorn sheep populations. The initial source of infection for this herd is unknown. Previous studies have suggested that bighorn sheep may be able to maintain Map within a population without ongoing introductions and, experimentally, Map of bighorn sheep origin can infect a range of other wild ungulates (Williams et al., 1983b). However, JD is equally an important disease in cattle across Alberta (Scott et al., 2001; Sorenson et al., 2003), and investigation into transmission patterns of Map between wildlife and livestock will be important in formulating recommendations for management of the potential risk to various host species. Genotyping, as performed in this study, is an essential first step to evaluating the likelihood, risks, and implications of interspecies transmission among bighorn sheep and other domestic and wild species. These baseline data will be useful for future strain-comparison studies with other regional wildlife and livestock species. Understanding the epidemiology and effects of Map in wild bighorn sheep populations is critical to understanding pathogen dynamics, evaluating translocation risks and conservation plans, and managing the wildlife-domestic animal interface.

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