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HEALTH STATUS OF REINTRODUCED WOOD BISON (*BISON BISON ATHABASCAE*): ASSESSING THE CONSERVATION VALUE OF AN ISOLATED POPULATION IN NORTHWESTERN CANADA

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ABSTRACT: A central goal for reintroduced populations of threatened wood bison (*Bison bison athabascae*) is to maintain them free of diseases of concern, particularly bovine tuberculosis (caused by *Mycobacterium bovis*) and brucellosis (caused by *Brucella abortus*). A wood bison population in southwestern Yukon, Canada was reintroduced into the wild in 1988, but no health assessment has been done since then. To provide an initial assessment of the health status and, hence, the conservation value of this population, we serologically tested 31 wood bison (approximately 3% of the population) for pathogens of interest and obtained histopathology results for select tissues. We found no evidence of exposure to *M. bovis* or *Brucella* spp., but antibodies were present to bovine parainfluenza virus 3, bovine coronavirus, *Leptospira interrogans*, and *Neospora caninum*, with seroprevalences of 87, 7, 61, and 7% of the tested animals, respectively. Reintroduced wood bison in southwestern Yukon may be of high value for wood bison recovery because it is a large and geographically isolated population with no bacteriologic, histopathologic, or serologic evidence of exposure to *Brucella* spp. or *M. bovis*.

Key words: *Bison bison*, *Brucella abortus*, health surveillance, *Leptospira interrogans*, *Mycobacterium bovis*, *Neospora caninum*, reintroduction, serology.

INTRODUCTION

Wood bison (*Bison bison athabascae*) declined precipitously in the late 1800s but were saved from extinction through concerted effort (Sanderson et al. 2008). Reintroduction of wood bison to their historical range has figured prominently in their restoration, and currently 11 reintroduced populations occur in northwestern Canada and Alaska. Remnant populations also occur primarily in and adjacent to Wood Buffalo National Park (WBNP), Canada. However, remnant populations contain animals that are infected with *Mycobacterium bovis* and *Brucella abortus*, limiting their conservation value (Shury et al. 2015). Extralimital movements, range expansion, and population connectivity are actively discouraged due to concern that these animals may transmit these diseases to livestock or to other reintroduced bison populations (Nishi et al. 2002; Wobeser 2009; Jung 2017).

Considerable effort has been expended to understand the pathogenesis, significance, and management of *M. bovis* and *B. abortus* in bison (Choquette et al. 1978; Rhyan et al. 2001; Joly and Messier 2004). Efforts to treat or eradicate wood bison infected with *M. bovis* and *B. abortus* are difficult because of the technical challenges in diagnosing and eliminating these diseases in free-ranging populations in remote landscapes (Himsworth et al. 2010; Shury et al. 2015). Although these pathogens have affected wood bison populations since the 1920s (Tessaro 1989), wood bison infected with *M. bovis* and *B. abortus* are largely confined to WBNP and the adjacent Slave River lowlands in the Northwest Territories and Alberta (Nishi et al. 2006; Wobeser 2009; Shury et al. 2015). Management strategies that focused on geographic

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isolation between populations have been the most effective method of preventing pathogen transmission between wood bison populations or to livestock (Shury et al. 2015); however, connectivity of populations is the long-term goal for wood bison recovery (Environment and Climate Change Canada 2016). Bovine tuberculosis and brucellosis are the most important limitation to successful recovery of wood bison and, accordingly, a central goal of the Canadian recovery strategy for wood bison is to maintain reintroduced populations free of these diseases (Environment and Climate Change Canada 2016).

One of the largest populations of reintroduced wood bison occurs in southwestern Yukon, Canada (the Aishihik Herd). The Aishihik Herd was reintroduced from stock that could be traced back to Elk Island National Park (Jung et al. 2015a, b; Clark et al. 2016). Wood bison in Elk Island National Park are tested annually for *M. bovis* and *B. abortus* and the population was declared free of these pathogens as of 1971 (Nishi et al. 2002), but wood bison imported into Yukon were not specifically tested before being translocated. From 1988 to 1993, 170 bison were released into the wild to establish the Aishihik Herd (Jung et al. 2015b), which was estimated to number 1,258 adults by July 2016 (T.S.J. unpubl. data). The Aishihik Herd is located approximately 1,200 km west of diseased populations in WBNP, effectively isolating it from wood bison populations infected with *M. bovis* and *B. abortus*. The nearest reintroduced population (Nordquist Herd) is approximately 500 km away in northeastern British Columbia (Shury et al. 2015). Its large population size and geographic separation from diseased populations make the Aishihik Herd a potentially important reservoir for wood bison restoration. While testing for diseases of concern is a recommended action in the management plan for the Aishihik Herd (Government of Yukon 2012), disease surveillance had not been done since it was reintroduced. We provide an initial assessment of the health status of the Aishihik Herd and, hence, its conservation value, with a focus on testing for the presence of *M. bovis* and *Brucella* spp. using serology, culture, and histopathology. In addition, we tested for serologic evidence of exposure to a range of pathogens.

MATERIALS AND METHODS

Sample collection

From 2014–17, we harvested and sampled 31 adult wood bison (26 females, 5 males) from the Aishihik Herd. We harvested and sampled 6–9 wood bison each April. We tried to focus on mature (≥6 yr old) females; however, age was only determined by cementum analysis after selection. Sampling effort was distributed to include individuals from different parts of the population's range, and mature males and females were spatially segregated during late winter (Jung 2015). Animals were shot from a helicopter after a ≤2 min aerial pursuit. They were transported up to 55 km by helicopter and truck to a facility in Haines Junction, Yukon, for necropsy and sample collection.

Blood was collected prior to transport from a cut-down of the jugular vein within 3 min of the animal's death. Blood was held between 10–18 C for ≤3 hr before it was centrifuged at approximately 3,430 × G for 10 min. Serum was removed and aliquoted into 1-mL cryovials (Nalgene Nunc International, Rochester, New York, USA) and immediately frozen at –20 C. After transport to the facility, we sexed and weighed the animal, took basic morphologic measurements, measured back fat, and collected an incisor for aging through cementum analysis (Moffitt 1998). Females were classified as reproductive if they had a fetus in utero at the time of death or had no fetus but were lactating.

We collected a metatarsal bone, both kidneys, various lymph nodes, and other tissues (i.e., tonsil, spleen, kidney, lung, liver, jejunum, ileum, and cecum, and ovary, testicle, mammary gland, placenta, uterus, and fetal stomach contents where applicable). Lymph nodes collected included submandibular, retropharyngeal, parotid, bronchial, mediastinal, prescapular, prefemoral, popliteal, supramammary, cervical, hepatic, mesenteric, ileocecal, and iliac. Duplicate sets of tissue samples from each bison were stored frozen at –20 C and fixed in 10% neutral-buffered formalin. Back fat was measured by making a small cut into the skin and subcutaneous tissue approximately 10 cm cranial to the tailhead and 10 cm lateral to the tip of the dorsal processes and using a ruler to measure the depth of subcutaneous adipose tissue. Bone marrow was extracted from the metatarsal bone by cutting the bone in half along the transverse midline, which was held at room temperature and weighed weekly until

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the weight of the dried bone marrow remained constant for at least 7 d. The bone marrow percent fat was calculated as: final weight/original weight (Ransom 1965). Frozen kidneys were thawed and weighed, fat was removed, and a kidney fat index was calculated as kidney weight without fat/kidney weight with fat (Ransom 1965).

Serum testing and analysis

Sera (n=31) were analyzed for the presence of antibodies to both *Brucella* spp. and *M. bovis* at the Canadian Food Inspection Agency (CFIA), Ottawa Animal Health Laboratory (Nepean, Ontario, Canada). A competitive enzyme-linked immunosorbent assay (C-ELISA) that was developed (Nielsen et al. 1995) and validated for use in bison (Gall et al. 2000) was used to test serum samples for antibodies to *Brucella* spp. Antibodies to *M. bovis* were measured with a fluorescence polarization assay (FPA; Surujballi et al. 2002) as modified by Jolley et al. (2007) using a 96-well platform assay format. Duplicate positive and negative bovine control antisera were included in every test. The mean of the millipolarization unit (mP) values obtained for the two negative sera was subtracted from the mP value obtained for each test serum, producing a delta mP value. The cut-off threshold value used for determining a positive result was delta mP value $\geq$20. This assay is validated by the CFIA for use in cattle (*Bos taurus*) but not in wood bison.

The presence of antibodies against bovine respiratory syncytial virus (BRSV), bovine herpesvirus-1 (BoHV-1), bovine parainfluenza virus 3 (PI3), and bovine coronavirus (BCV) were assessed with a bovine respiratory ELISA panel (Durham and Hassard 1990) in a commercial laboratory (Prairie Diagnostic Services, Saskatoon, Saskatchewan, Canada). Samples that were categorized as suspicious by the testing laboratory (reference range=3–13 ELISA units for BoHV-1 and 11–13 ELISA units for BCV) were classified as negative. Testing for antibodies to *Neospora caninum* was conducted by the same laboratory with a commercial kit (*N. caninum* Antibody Test Kit, C-ELISA), as per manufacturer's directions (Veterinary Medical Research and Development Inc., Pullman, Washington, USA).

Serum samples were tested for antibodies against bovine viral diarrhea virus (BVDV) type 1 (with NADL strain; BVDV-1) and type 2 (with NVSL 125c strain; BVDV-2) using a virus neutralization test in a commercial laboratory (Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada). Briefly, sera were heated in a water bath at 56±2 C to destroy complement, diluted in duplicate in media as doubling dilutions, and incubated for 1–1.5 hr at 5±3 C with 100 cell culture infective doses of virus to allow an antigen-antibody reaction to occur. Subsequently, cells were added and the plates incubated at 37±2 C in 5% carbon dioxide gas to allow replication of any virus that was not neutralized by antibody in the serum. At the end of the 4–5 d incubation period, each well was inspected for cytopathic effect using an inverted microscope. The end-point was defined as the highest dilution of serum which showed at least 50% cytopathic effect, and the titer was the reciprocal of the dilution. Serum samples with a titer $\geq$2 were considered positive for BVDV.

Serum samples were also tested at the same laboratory as mentioned earlier for antibodies against seven *Leptospira interrogans* serovars (namely, Autumnalis [strain Akiyami A, representing serogroup Autumnalis], Bratislava [strain Jez Bratislava, serogroup Australis], Canicola [strain Hond Utrecht IV, serogroup Canicola], Grippotyphosa [strain Moskva V, serogroup Grippotyphosa], Hardjo [strain Hardjoprajitno, serogroup Sejroe], Icterohaemorrhagiae [strain M20, serogroup Icterohaemorrhagiae], and Pomona [strain Pomona, serogroup Pomona]) using a microscopic agglutination test. Samples were first screened at 1:50 dilution, and then endpoint titers were determined for any samples that gave a reaction at that dilution. The end-point was defined as the highest dilution of serum which showed at least 50% agglutination, with the titer reported as the reciprocal of that dilution. Serum samples with a titer of 100 or higher were considered positive. Cultures and reference antisera were obtained from the US Department of Agriculture (USDA) Animal Plant Health Inspection Service (National Veterinary Services Laboratories, Ames, Iowa, USA) and tests were performed as recommended by international health authorities (World Health Organization and International Leptospirosis Society 2003). The methods used for serum analysis described for the detection of BRSV, BoHV-1, PI3, BCV, *N. caninum*, BVDV-1, BVDV-2, and *L. interrogans* have not been validated for use in wood bison but are used routinely in cattle (Durham and Hassard 1990; Baszler et al. 2001; Van de Weyer et al. 2011; Dubovi 2013).

Histopathologic examination and bacterial culture

Histopathologic examination and bacterial culture were performed at the CFIA Animal Health Laboratory. Formalin-fixed tissues were processed routinely, embedded in paraffin, sectioned at 4 μm, and stained with H&E and Ziehl-Neelsen modified acid-fast stains (Luna 2008) for histologic examination.

Frozen tissues were thawed and examined grossly for lesions consistent with *Mycobacterium* spp. Approximately 1–3 g of tissue (preferably

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tissue sections containing lesions) were pooled according to the anatomic location (i.e., head, thorax, and abdomen). The pools were homogenized (Kinematica Polytron, Switzerland) and decontaminated using sodium hydroxide treatment before inoculation for routine mycobacterial isolation on Herrolds medium with mycobactin and antibiotics, on Lowenstein Jensen medium with glycerol, Lowenstein Jensen medium with pyruvate, Modified Middlebrook with Malachite Green, Middlebrook 7H9 with antibiotics, Middlebrook 7H10 medium with mycobactin and a *Mycobacteria* Growth Indicator Tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), and Stonebrink medium (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Inoculated media were incubated at 37 C and examined for growth once a week for 2 wk and then once every 2 wk for up to 10 wk.

To culture and identify *Brucella* spp., tissue samples were arranged into pools based on anatomic location as above, cut into smaller sections, and homogenized using a Stomacher blender (Seward; Worthing, West Sussex, UK) for 5 min. Contents of the Stomacher bag were then filtered through the median membrane into 50-mL centrifuge tubes and used in inoculation of blood agar medium, USDA Brucella agar medium, World Health Organization Brucella agar medium, USDA Brucella agar medium plus ethyl violet, Serum-dextrose agar (Becton, Dickinson and Company), Oxoid Brucella medium, Oxoid Brucella Selective medium, and Oxoid Brucella Modified Selective medium (Oxoid Company, Nepean, Ontario, Canada). The plates were incubated in 5–10% carbon dioxide at 37 C and read at day 3 or 4 and then at days 7 and 14.

RESULTS

Sampled bison

We collected samples from 31 bison including five males and 26 females. Most were $\geq$4 yr old, but seven individuals were $\leq$3 yr old including three females. Mass and measures of body condition (back fat, bone marrow, kidney fat index) varied widely among individuals. We found no significant differences ($P \geq 0.123$) between these groups with respect to age, mass, back fat, bone marrow, or kidney fat index (Table 1).

Histopathologic examination

The histopathologic lesions found in animals of the Aishihik Herd were mild and incidental overall. The tissue sections examined showed no evidence of mycobacteriosis or brucellosis. One bison (3%, 1/31) displayed the following lesions: mild focal lymphocytic perivasculitis within the heart; mild hepatic periportal lymphocyte infiltration; moderate sinusoidal neutrophilia and eosinophilia of a prescapular lymph node; and a splenic siderotic plaque. Two animals (7%, 2/31) displayed mild lymphoplasmacytic peribronchial aggregates, and one of these animals also had patchy eosinophil exocytosis through the bronchial epithelium. Three bison (10%, 3/31) each presented with single granulomas within a tonsil, the cecum, and submandibular lymph node. One animal (3%, 1/31) had two granulomas; one in the perinodal fat of the hepatic lymph node and one within the capsule of an ileocecal lymph node. Generally, the granulomas were associated with multinucleated giant cells and few eosinophils. Three of the five granulomas could not be reidentified with recuts, and no acid-fast bacilli were identified with Ziehl-Neelson modified acid-fast staining. None of the observed granulomas were considered compatible with *M. bovis*.

Three (10%) animals displayed moderate to severe, diffuse hepatic lipidosis. Tonsillar crypt abscesses, splenic hemosiderosis, and mineralization of renal collecting tubules were common findings among sampled bison. There was variable distention of the tonsillar crypts by degenerate cell debris, desquamated epithelial cells, bacteria, and plant material in most animals. Many animals had mild, focal interstitial lymphocytic aggregates in the kidney. Mild eosinophilic capsulitis, mild sinusoidal neutrophilia, and variable sinusoidal histiocytosis associated with hemosiderin pigment or yellow-brown crystalline foreign material was commonly observed within lymph nodes. Additionally, there was variable infiltration of the intestinal lamina propria by eosinophils and in a few animals the proprial lymphoplasmacytic infiltrate was prominent.

Serology

None of the 31 sampled bison had antibodies detectable by the tests for *Brucella* spp.,

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TABLE 1. Samples sizes and mean (±SD) age, mass, and body condition measures (back fat, bone marrow, kidney fat index) for 31 adult wood bison (*Bison bison athabascae*) harvested and sampled for serology and serum biochemistry in southwestern Yukon, Canada, 2014–17. Test statistics reported are Kruskal-Wallis tests across sample groups.

Parameter	Treatment group			Test statistics	
	Males (n=5)	Reproductive females (n=21)	Nonreproductive females (n=5)	H	P
Age	8.4±3.2	5.4±3.3	6.6±5.2	4.18	0.123
Mass (kg)	513±145	487±67[a]	443±186	0.18	0.915
Back fat (mm)	2.4±1.9	5.1±3.5	3.6±3.0	2.79	0.248
Bone marrow (% fat)	81.7±5.1	77.5±20.65	86.9±4.5	1.36	0.506
Kidney fat index	51.4±17.2	51.3±15.8	35.7±7.9	5.13	0.077

[a] $n = 20$.

M. bovis, BRSV, BoHV-1, BVDV-1, or BVDV-2 (Table 2). Most of the sampled animals (87%, 27/31) had antibodies to PI3, and a low number (2/31, 7% each) had antibodies to BCV and *N. caninum*. For bison that were seropositive for *L. interrogans* antibodies (61%, 19/31), seven different serovars were found. Bratislava (36%, 11/31) and Icterohaemorrhagicae (36%, 11/31) were the most-commonly detected serovars followed by Canicola (23%, 7/31), Pomona (16%, 5/31), Hardjo (10%, 3/31), Autumnalis (7%, 2/31), and Grippotyphosa (3%, 1/31). Eight animals were seropositive for one leptospiral serovar, four were seropositive for two, four were seropositive for three, two were seropositive for four, and one animal was seropositive for six serovars.

DISCUSSION

Histopathologic examination

The majority of the histopathologic findings in the Aishihik Herd were clinically insignificant. The focal granulomas found within the lymph nodes, tonsil, and cecum of some animals were presumed to be associated with parasitic infestations, given the presence of eosinophils. Other findings included splenic hemosiderosis and tonsillar abscesses. Splenic hemosiderosis is an accumulation of hemosiderin, an iron derivative from erythrocyte metabolism, in the spleen. This is usually clinically insignificant in most species, as it is the by-product of normal erythrocyte metabolism (Cullen 2007; Fry and McGavin 2007) and has been reported as normal in bison (Zaugg et al. 1993). Splenic siderotic plaques are focal accumulations of hemosiderin found as dry, hardened nodules on the splenic capsule (Fry and McGavin 2007). In domestic animals, they are associated with age or previous hemorrhage and were considered incidental in the bison in which they were found (Fry and McGavin 2007). The tonsillar abscesses were likely due to a foreign body or a minor secondary bacterial infection and the body's inflammatory response to prevent their further spread.

Serologic tests

We found no serologic evidence of infection or exposure to *Brucella* spp. in 31 animals (about 3% of the adult population) sampled over 4 yr from the Aishihik Herd, using an ELISA that has been validated for use in bison with relatively high estimates of sensitivity (92%) and specificity (98%; Gall et al. 2000). This finding has been corroborated by the *Brucella*-negative results obtained from the bacteriologic culture of a comprehensive list of tissues from these animals. Animals infected with *M. bovis* initially develop a robust cell-mediated immune response to the pathogen followed by a humoral immune response (Fifis et al. 1994). Diagnostic tools such as the skin test or the gamma interferon test, which measure elements of the cell-

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TABLE 2. Results for reintroduced wood bison (*Bison bison athabascae*; *n*=31) tested for *Brucella* spp., *Mycobacterium bovis*, and other pathogens in southwestern Yukon, Canada, 2014–17.

Disease agent	Test method[a]	No. positive/no. tested	% Positive
Brucella spp.	C-ELISA[b]	0/31	0
Brucella spp.	Culture[c]	0/31	0
Mycobacterium bovis	FPA[b]	0/31	0
Mycobacterium bovis	Culture[c]	0/31	0
Bovine respiratory and syncytial virus	ELISA[b]	0/31	0
Bovine herpesvirus	ELISA[b]	0/31	0
Bovine parainfluenza 3	ELISA[b]	27/31	87.1
Bovine coronavirus	ELISA[b]	2/31	6.5
Bovine viral diarrhea virus 1	Serum neutralization[b]	0/31	0
Bovine viral diarrhea virus 2	Serum neutralization[b]	0/31	0
Leptospira interrogans	MAT[b]	19/31	61.3
Neospora caninum	C-ELISA[b]	2/31	6.5

[a] C-ELISA = competitive enzyme linked immunosorbent assay; FPA = fluorescence polarization assay; ELISA = enzyme linked immunosorbent assay; MAT = microscopic agglutination test.

[b] Sample was serum.

[c] Sample was tissue.

mediated immune response as correlates of infection with this pathogen, are in predominant use. However, it was not possible to use the skin test, given that the animals used in this study were killed for sample collection. The gamma interferon test was not used as it was not possible to ship whole blood to the laboratory in a timely fashion, given the remote location of the Aishihik Herd. Serologic tests are being increasingly used to monitor such infections in a variety of animal species but, to our knowledge, none have been validated for use in bison. Nevertheless, a serologic test offers a feasible alternative for monitoring bovine tuberculosis because serum can be obtained and preserved relatively easily. However, in the absence of test validation data in bison, results need to be interpreted with caution. In this study, we employed a FPA that uses an antigen derived from the MPB70 protein of *M. bovis* and, as such, its sensitivity is limited because antibodies elicited against additional *M. bovis* antigens such as MPB83, ESAT-6, and CFP10 will potentially go undetected. The primary reason for using the FPA is that this test does not require a species-specific reporting system. Because we used a cut-off threshold that was developed for use in cattle for classifying a result as positive, the results of this test do not indicate the presence of antibody to the MPB70 antigen in the 31 bison examined. This interpretation of the serologic results is supported by the histopathologic and bacteriologic culture data which indicate that none of these animals were infected with *M. bovis*.

The high prevalence of antibodies to bovine PI3 in wood bison from the Aishihik Herd (87%) was similar to that reported from free-ranging populations of plains bison (*Bison bison bison*) in Alaska (99%, 216/281 bison sampled between 1983–88; Zarnke and Erickson 1990) and Wyoming (100%, 16/16; Williams et al. 1993), although Taylor et al. (1997) presented a lower prevalence (36%, 27/75) for those in Yellowstone National Park. It is presumed that PI3 was introduced to plains bison in Alaska from domestic cattle (Zarnke and Erickson 1990), with which they comingled. The origin of PI3 in the Aishihik Herd is unknown, and to the best of our knowledge these animals do not interact with cattle or other domestic livestock; however, it is possible that the founding population was exposed to the virus prior to reintroduction. Regardless of its origin in the Aishihik Herd, Karstad (1981) considered that PI3 is an incidental finding in most species of wildlife

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while others concluded that the high percentage of animals seropositive for PI3 does not appear to be clinically significant for free-ranging bison populations (Zarnke and Erickson 1990; Taylor et al. 1997).

The Aishihik Herd had a low seroprevalence of BCV (7%, 2/31). Both of the positive bison were collected in 2014. Bovine coronavirus is characterized as a severe enteric pathogen in cattle worldwide (Clark 1993). A bovine-like coronavirus was recently identified in a European bison (*Bison bonasus*) during a dysentery outbreak at a Korean zoo (Chung et al. 2011). Bovine coronavirus has otherwise not been documented in American bison. Various coronaviruses are commonly identified in ungulates, but the epidemiology of these viruses in free-ranging populations is not yet well understood (Evermann and Benfield 2001). It has been suggested that serology is not the most accurate method of detection of coronaviruses due to low antibody titers (Evermann and Benfield 2001; Gilbert et al. 2013). Overall, it is unlikely that BCV is a highly prevalent or major pathogen of concern in the Aishihik Herd.

Seroprevalence of *N. caninum* in animals we sampled from the Aishihik Herd was low (7%, 2/31). Both of the positive bison were collected in 2016. A low prevalence of *N. caninum* was similarly reported for plains bison (2%, 5/249; Dubey and Thulliez 2005) and for European bison tested by Cabaj et al. (2005; 7%, 23/320) and Bień et al. (2010; 13%, 3/23). This parasite has been successfully isolated from the peripheral blood of a European bison (Bień et al. 2010) but not from the animals in which antibody presence was detected in northern Canada (Kutz et al. 2012). Stieve et al. (2010) attributed a low *N. caninum* seroprevalence across several cervid and canid species tested in Alaska to low densities of definitive hosts and cold winter temperatures limiting the survival of oocysts in the environment. Although stress may exacerbate the effects of neosporosis on an intermediate host, it is not well understood what clinical effects this pathogen has on bison (Shoemaker 2014). Given the low densities of agricultural species in northern Canada, it is more likely that *N. caninum* maintains a sylvatic life cycle rather than having been introduced to wildlife from livestock (Kutz et al. 2012).

Positive antibody titers to *L. interrogans* were relatively common (61.3%) in sampled wood bison from the Aishihik Herd. Prevalence in this population was higher than that reported for any other population of bison tested: for example, *L. interrogans* antibodies were not detected in plains bison in Kansas (Williams et al. 1993) but were found at prevalences of 5% in 1990 and 7% in 1993 in Alaska (Zarnke and Erickson 1990) and 16% in Yellowstone National Park (Taylor et al. 1997). Prevalences similar to what we found for the Aishihik Herd were reported for plains bison (57%; Vestweber et al. 1991) and European bison (58%, 35/60; Kita and Anusz 1991). When separated by serovars, Taylor et al. (1997) reported the following prevalences: 4% for *L. i.* Icterohaemorrhagicae, 7% for *L. i.* Hardjo, 1% for *L. i.* Autumnalis, 7% for *L. i.* Bratislava, and 1% *L. i.* Australis. All of the serovar prevalences reported by Taylor et al. (1997) are substantially lower than the serovar prevalences found in bison we sampled, particularly with respect to *L. i.* Bratislava and *L. i.* Icterohaemorrhagicae, which both had prevalences of 36% in the Aishihik Herd.

Maintained in the renal system of its host, *L. interrogans* can be transmitted between suitable hosts through contact with urine-contaminated forage or water bodies. Leptospirosis is considered a worldwide re-emerging zoonotic disease in both rural and urban environments, and its increasing prevalence is believed to be linked to changing patterns in precipitation due to climate change (Lau et al. 2010). Although generally considered a pathogen of temperate regions, *L. interrogans* has been identified in human infections in northern Quebec (Messier et al. 2012). In Canada, it is increasingly becoming a pathogen of concern (Levett 2004). However, as far as we know, these are the first reported incidences of exposure to *L. interrogans* in wildlife in northwestern Canada.

In bison, leptospirosis can cause abortions in the last half of pregnancy (Kipps 1990).

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Additionally, it can cause chronic renal and reproductive failure (Cook and Karesh 2012). In many species, *L. i.* Icterohaemorrahagicae is associated with severe clinical disease (Adler and de la Peña Moctezuma 2010). However, no lesions consistent with leptospirosis were present in the bison we sampled. The bison sampled by Kita and Anusz (1991) also lacked additional clinical signs, and they concluded that those animals were not truly infected with leptospirosis. Interestingly, rats (*Rattus norvegicus* and *Rattus rattus*) are considered the maintenance hosts of *L. i.* Icterohaemorrhagicae (Levett 2004), but rats are not known from rural Yukon (Jung et al. 2011). Horses (*Equus caballus)* and pigs (*Sus scrofa*) are most-commonly affected by *L. i.* Bratislava (Adler and de la Peña Moctezuma 2010). It is unknown what maintenance host species are responsible for the exposure of the Aishihik Herd to *L. interrogans* and to serovars Icterohaemmorhagicae and Bratislava in particular. Further studies are necessary to understand the clinical significance of *L. interrogans* on wood bison and the role that bison play in its maintenance in the ecosystem.

In conclusion, we report exposure of wood bison to *L. interrogans* and suggest further investigation into the prevalence of this pathogen in northern wildlife and the impact that it may have with respect to wood bison recovery. However, our main finding is that, based on histopathology, bacteriologic culture, and serology results, there is no initial evidence that the Aishihik Herd has been exposed to, or is infected with, *Brucella* spp. or *M. bovis*. This is significant because brucellosis and bovine tuberculosis are the main impediments to wood bison restoration (Shury et al. 2015).

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