Research Article

Lesion Distribution and Epidemiology of *Mycobacterium bovis* in Elk and White-Tailed Deer in South-Western Manitoba, Canada

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Surveillance for *Mycobacterium bovis* in free-ranging elk (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*) from south-western Manitoba was carried out from 1997 to 2010 to describe the lesions, epidemiology, and geographic distribution of disease. Tissues were cultured from animals killed by hunters, culled for management, blood-tested, or found opportunistically. Period prevalence in elk was approximately six times higher than deer, suggesting a significant reservoir role for elk, but that infected deer may also be involved. Prevalence was consistently higher in elk compared to deer in a small core area and prevalence declines since 2003 are likely due to a combination of management factors instituted during that time. Older age classes and animals sampled from the core area were at significantly higher risk of being culture positive. Positive elk and deer were more likely to be found through blood testing, opportunistic surveillance, and culling compared to hunting. No non-lesioned, culture-positive elk were detected in this study compared to previous studies in red deer.

1. Introduction

Riding Mountain National Park (RMNP) is a 2974-hectare protected area that is part of a large elevated escarpment and is part of a UNESCO (United Nations Educational, Scientific and Cultural Organization) Biosphere Reserve. This area, which includes the Duck Mountain Provincial Park and Forest (DMPPF) is an important core habitat for a large population of elk (*Cervus elaphus)*, moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), wolves (*Canis lupus*), and black bears (*Ursus americanus*) and is considered a southern extension of the boreal forest in Canada. Both protected areas are essentially surrounded on all sides by agricultural landscapes which include forage crop production, grain farming, and livestock production. Cattle were grazed sympatrically with wildlife within RMNP and the DMPPF until 1970 when cattle grazing was discontinued in both areas [1]. Fourteen cattle herds have been found to be infected with bovine tuberculosis (bTB) since 1991 in the area around RMNP, and several of these have been closely linked to cases of infected deer and elk [2, 3].

Local cattle producers have been involved with intensive live cattle testing and movement restrictions, resulting in negative economic consequences for these producers. The two Manitoba Game Hunting Areas that surround RMNP were designated a special management zone called the Riding Mountain Eradication Area (RMEA) in 2003. Following extensive live cattle testing for three years, cattle herds within this zone were subsequently deemed to be TB-free according to Canadian livestock standards in August of 2006. One additional herd breakdown in cattle was found within the RMEA in May of 2008 [1], but no infected cattle herds have been discovered after extensive follow-up testing since that time. All *M. bovis* isolates to date from cattle, deer, and elk share two closely related spoligotypes designated MB-1 and MB-2 including two infected wolves found within RMNP in 1978 [4, 5]. It is likely that wildlife species were initially infected as a result of contact with infected cattle, but the infection has likely spilled back to cattle since that time.

Emerging wildlife reservoirs of *M. bovis* infection have created serious negative socioeconomic consequences in the past 15 years in Europe, North America, and New Zealand,

particularly when the wildlife reservoir has significant conservation or societal value [6]. Determination of disease burden and of species acting as reservoirs is particularly challenging with infected wildlife populations. Reservoir hosts for *M. bovis* are those species that can maintain infection independently through intraspecific transmission without reinfection from another species, while spillover hosts require reinfection from another species to maintain the infection and typically do not maintain the infection in wild populations [6, 7]. Some species may act as both reservoir and spillover hosts depending on demographic and population-specific factors such as population density, presence of artificial feeding, and host immunity [8–10] and species may form reservoirs in combination [9]. In North America, white-tailed deer have been demonstrated to be a competent reservoir species in Michigan, USA while elk are considered a spillover host [8, 11]. A separate, unrelated outbreak of *M. bovis* is currently occurring in white-tailed deer in the state of Minnesota, but the disease does not appear to be spreading rapidly and deer-to-deer transmission may not be occurring in this state [12]. The epidemiology of bovine TB has been described for wild red deer in New Zealand [13, 14] and Spain [15–17], but very few references describe the epidemiology or prevalence in wild elk from North America [2, 8]. The enzootic described in this paper is even more challenging from a disease control perspective as the wildlife that make up the likely reservoir species are found within two environmentally sensitive protected areas (RMNP and DMPPF). Hunting or direct culling have typically been used as a management tools to control wildlife host density and provide samples for disease surveillance, but hunting is not currently permitted within RMNP, making disease management at a landscape scale extremely challenging [1, 17, 18]. This area is one of the last known reservoirs of *M. bovis* in Canada [18], and little is known about the status of this infection in elk and deer in this area.

This study reports on preliminary pathologic findings, lesion distribution, and descriptive epidemiology from the area around RMNP and DMPPF for both white-tailed deer and elk and provides a brief analysis of *M. bovis* confirmed cases found since 1997 in this area. Prevalence and distribution data will be presented allowing a comprehensive assessment of this long-term wildlife reservoir and a discussion of implications for future management and eradication of the disease in wildlife.

2. Methods and Materials

2.1. Sample Collection. *Mycobacterium bovis* infection was initially discovered in wild ungulates from the RMNP area in a hunter-killed bull elk in 1992, but formal surveys were not initiated until 1997 when hunter harvested elk were collected on the borders of RMNP [1, 2]. Data for this study includes deer and elk collected in the RMNP and DMPPF areas through four primary sources: (1) hunter-killed elk and deer collected as part of *M. bovis* surveillance efforts between November 1997 to January 2010 (hunter sample), (2) elk and deer collected as part of a blood testing

program within RMNP from February 2002 to May 2010 (blood test sample), (3) ground-based culls which were conducted to reduce elk and deer density and determine *M. bovis* prevalence in March 2004 (white-tailed deer only) and a February/March 2009 cull involving both elk and deer (cull sample), and (4) targeted surveillance samples which were collected opportunistically (roadkills, predation, and winter kills) and those animals destroyed because they were exhibiting clinical signs of illness (opportunistic sample). Hunter submissions typically consisted of both head and lung samples from harvested animals, but samples occasionally consisted of only the head or lungs. Blood testing was carried out through live animal capture and testing to detect antibodies and cell-mediated immunity to *M. bovis* (details provided below). A cull involving local landowners and Manitoba Conservation staff involving white-tailed deer was carried out in March of 2004 through ground-based shooting of deer in areas bordering RMNP. In 2009, culls for population reduction and surveillance were carried out within RMNP and involved helicopter net gun capture followed by euthanasia with captive bolt gun. All culled animals were transported intact to a laboratory where a full necropsy was conducted on each carcass. Head and lung samples from hunter killed animals were examined at the same laboratory (detail provided below). Targeted surveillance samples were collected opportunistically as a result of public reports and followup of predator kills for other research projects. White-tailed deer and elk were considered *M. bovis* positive if they were determined to have a positive culture on any tissue cultured for postmortem analysis.

Elk and deer captured for blood testing were primarily captured within two protected areas in south western Manitoba, Canada: RMNP and the DMPPF. Animal capture was carried out using helicopter net gunning between February 2002 and May of 2010 during winter and early spring (December to early June) (Figure 1). Elk were selected haphazardly by the helicopter crew in selected regions within RMNP and DMPPF, but virtually all elk and deer capture for blood testing occurred within these two protected areas. All captured elk were blindfolded and hobbled for short duration (10–15 minutes) and were released immediately after sampling and application of a VHF or GPS collar to allow subsequent relocation and recapture. A cotton spacer made of fire hose was attached to the collar belting to cause them to fall off within 3–6 months after capture. Sixty millilitres of whole blood was collected by jugular venipuncture and placed in either 10 mL sterile glass vials containing no additive, lithium heparin (Vacutainer), or silicone coating (Vacutainer SST). Samples without anticoagulant were allowed to clot at room temperature and centrifuged at 3,000 rpm for 15 minutes. For the period 2004 to 2010, three blood-based assays were used to detect potentially infected cervids; a lymphocyte stimulation test (LST), a fluorescence polarization assay (FPA) [19], and a chromatographic immunoassay (Cervid Stat-Pak) [20]. An experimental polymerase chain reaction (PCR) test was also utilized on buffy coat samples in 2002 to 2004 in addition to these three tests, but it was discontinued in 2005. Serum
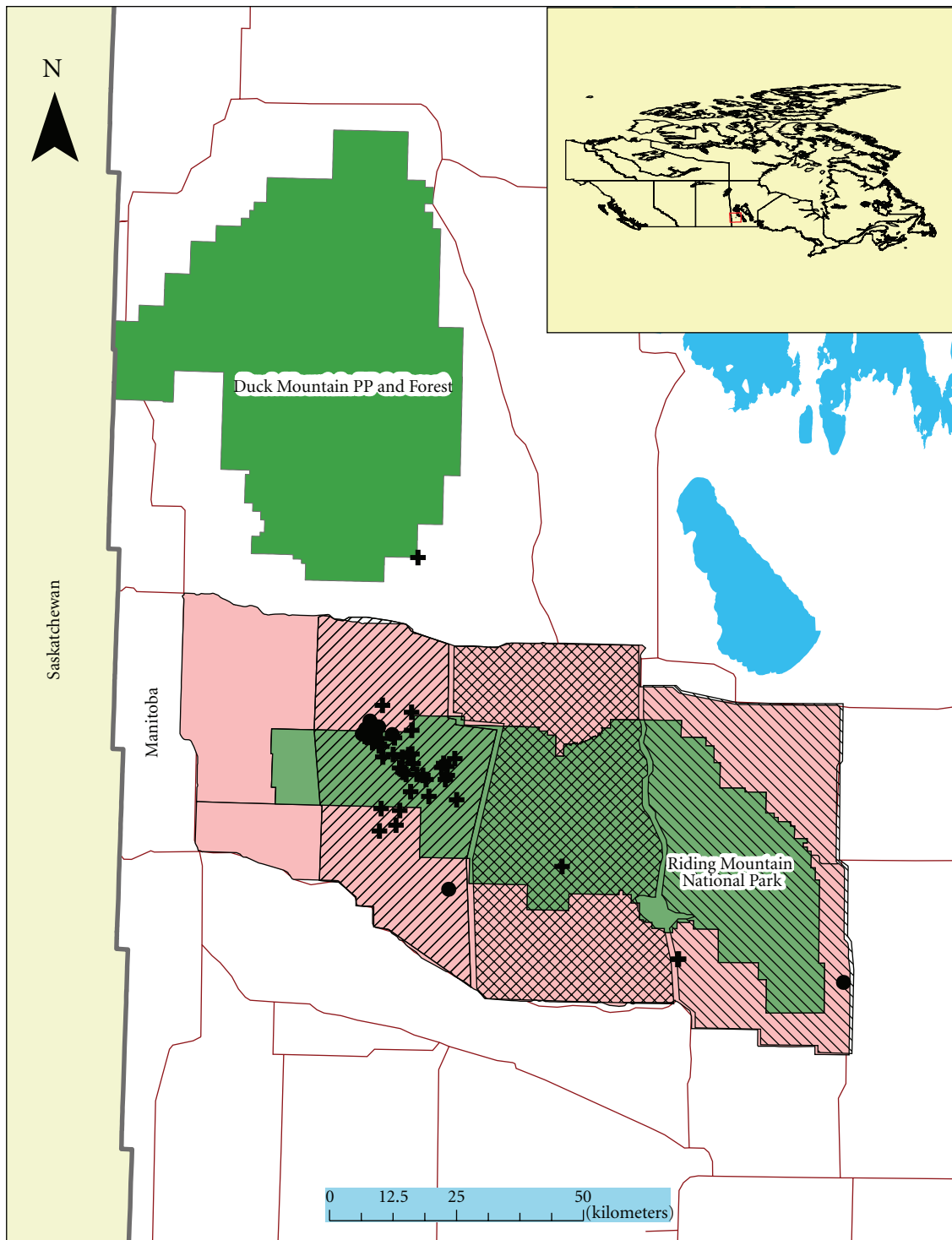
Figure 1: Locations of sampling zones and *M. bovis* culture positive elk and deer cases in south-western Manitoba from 1997 to 2010.

for the Cervid Stat-Pak evaluation was harvested and frozen at −20°C or tested immediately in some cases. Fresh whole blood with and without anticoagulant were stored at room temperature and shipped immediately upon collection to the Canadian Food Inspection Agency, Mycobacterial Diseases Centre of Expertise (MDCE), Ottawa, Ontario for evaluation using the LST and FPA, respectively. Elk testing positive (parallel interpretation) on any one of these diagnostic tests (FPA, LST, and Stat-Pak) were subsequently recaptured up to two months later using the methodology described above, euthanized with a captive bolt gun and slung by helicopter to a central laboratory for immediate necropsy. Elk testing negative to three of the four tests (LST, FPA, and RT) were not recaptured, but were monitored by aerial telemetry until their radio collars fell off within 3–12 months after capture. A subset of animals that were culled and were tested retrospectively were used to validate the sensitivity (Se = 100%, 95% confidence intervals 56.5%–100%) of the parallel testing protocol, so very few of these animals were likely truly TB positive (unpublished data). Parallel testing involving multiple tests increases the sensitivity while sacrificing specificity, resulting in numerous false positives, but few false negatives [21].

Hunter sampled elk and white-tailed deer heads and lungs were collected annually between September 1997 and January 2010 from voluntary submissions by local hunters through regular and extended hunting seasons. Submissions have been mandatory since 1999 in the RMEA and since 2000 in the Duck Mountain Provincial Park and Forest. All submitted heads and lungs were examined grossly, with specific lymphoid tissues being sent for mycobacterial culture and histopathology prior to the fall/winter of 2001/2002. Since 2002, only tissues from animals exhibiting suspect gross lesions of tuberculosis in lymphoid tissues or palatine tonsils were submitted for histopathology and mycobacterial culture. Hunting is not allowed within RMNP, but elk and white-tailed deer hunting are allowed within the DMPPF and surrounding area (Figure 1). A set of four lymph nodes were routinely evaluated in the head (medial retropharyngeal, parotid, submandibular, and lateral retropharyngeal) as well as the palatine tonsils. Lymphoid tissues were sliced thinly at 3–5 mm thickness to look for lesions typical of *M. bovis* and formalin-fixed tissue and fresh tissues were sent to the Mycobacterial Diseases Centre of Excellence (MDCE) laboratory in Ottawa, Ontario. Lung tissues were examined similarly with tracheo-bronchial and mediastinal lymph nodes being specifically targeted while lungs were palpated for abnormalities and sliced at 5 cm intervals to check for grossly visible lesions.

Elk and deer sampled opportunistically included predator killed animals, roadkilled animals, poaching investigations, winter killed animals, or animals observed with unusual clinical signs that were euthanized for necropsy. These animals were either necropsied in a laboratory or in the field depending on location.

2.2. Postmortem and Laboratory Procedures. For animals that tested positive on one or more blood tests and for the culled elk and deer, multiple tissues were collected at necropsy as part of a detailed postmortem collection procedure similar to that collected for other studies involving European badgers (*Meles meles*) [22] and subjected to mycobacterial culture, acid fast staining, and histopathological examination. Peripheral lymphoid tissues examined and collected were submandibular, medial, and lateral retropharyngeal, parotid, palatine tonsil (tonsillar crypt), prescapular, popliteal, prefemoral, supramammary/testicular, internal iliac, hepatic, portal, mesenteric, tracheo-bronchial, and mediastinal lymph nodes. Pools of tissue from body, head, abdominal, and thoracic lymph nodes were submitted for mycobacterial culture regardless of whether gross lesions were seen at necropsy or not. All other organ systems were systematically examined for gross lesions indicative of mycobacteriosis and any suspect tissue was also sent for mycobacterial culture, histopathological evaluation, and PCR testing to confirm identity of cultured mycobacteria. Harvested tissues were either frozen at −20°C or refrigerated and were shipped to the MDCE within 24 to 48 hours of collection. Formalin-fixed tissues were embedded in paraffin, cut into sections 5 mm thick, and stained with hematoxylin and eosin as well as by the Ziehl-Neelsen technique for detection of acid fast bacilli. Slides of the tissue sections were examined by a pathologist experienced in the diagnosis of TB. The tissues were cultured for mycobacteria using the method described by Rohonczy et al. [23]. Inoculated media were incubated at 37°C for 12 wk and examined every 2 weeks for evidence of bacterial growth. Elk and deer were considered TB positive if they had a positive culture for *M. bovis* on any tissue submitted for culture [19]. Spoligotyping to type cultured TB complex organisms was conducted as described previously [5]. Ages of hunter killed elk and deer at necropsy were determined by estimation of tooth wear into one of five age categories; less than one year of age, one to two years of age, three to five years of age, six to eight years of age, or greater than 8 years. Elk and deer that were culled, blood tested, or found opportunistically were aged by examination of tooth sections and counting cementum annuli [24].

2.3. Statistical Analysis. Sampled elk and deer were grouped based on sampling location into one of four risk zones created to monitor the prevalence and distribution of *M. bovis* in wildlife (Figure 1). Prevalence was estimated using the methods described in Thrusfield [21] and 95% confidence intervals were estimated using WINPEPI software version 10.1 using Wilson's score method [25]. Trend analysis on prevalence data was conducted using WINPEPI software using a two-way Cochrane-Armitage test for trend with Fishers exact 95% confidence intervals. Analyses of the proportion of culture positive animals with gross visible lesions in different tissues were compared using Upton's modified $(N − 1)$ Chi-square [26].

3. Results

The overall prevalence of *M. bovis* infection in elk and white-tailed deer has been consistently very low in the area

TABLE 1: Zone specific prevalence of *M. bovis* in elk and deer from south-western Manitoba from 1997 to 2010.

Species	Sampling Year[a]	Outside[b]			Eastern Surveillance Zone			Central Surveillance Zone			Western Control Zone		
		Prevalence (%)	95% CI	No. Tested	Prevalence (%)	95% CI	No. Tested	Prevalence (%)	95% CI	No. Tested	Prevalence (%)	95% CI	No. Tested
Elk	1997/1998	0	0–65.76	2	0	0–4.87	75	0	0–11.0	31	0	0–11.35	30
	1998/1999	0	0–13.32	25	0.81	0.14–4.43	124	1.72	0.31–9.1	58	0	0–5.13	71
	1999/2000	0	0–6.02	60	1.37	0.24–7.36	73	0	0–6.02	60	1.85	0.33–9.77	54
	2000/2001	0	0–3.26	114	0	0–2.12	177	0	0–3.47	107	2.99	1.29–6.82	167
	2001/2002	0	0–4.42	83	0	0–4.01	92	0	0–11.03	31	0.00	0–5.58	65
	2002/2003	0	0–2.96	126	0	0–2.26	166	0	0–6.02	60	6.85	3.76–12.15	146
	2003/2004	0	0–3.89	95	0	0–1.83	206	0	0–5.35	68	2.46	1.06–5.64	203
	2004/2005	0.55	0.1–3.05	182	0	0–4.28	86	0	0–15.5	21	3.01	1.18–7.48	133
	2005/2006	0	0–1.62	233	0	0–5.92	61	0	0–5.5	66	1.64	0.29–8.72	61
	2006/2007	0	0–2.87	130	0	0–4.87	75	0	0–11.35	30	3.16	1.08–8.88	95
	2007/2008	0	0–3.43	108	0	0–6.42	56	0	0–27.75	10	4.39	1.89–9.86	114
	2008/2009	0	0–4.53	81	0	0–25.9	11	0	0–27.75	10	1.09	0.3–3.88	184
	2009/2010	0	0–3.56	104	0	0–29.9	9	0	0–4.58	80	1.35	0.24–7.27	74
WTD[c]	1997/1998	0	–0	0	0	0–56.1	3	0	0–56.1	3	0	0–0	0
	1998/1999	0	0–5.5	66	0	0–7.41	48	0	0–12.1	28	0	0–29.9	9
	1999/2000	0	0–10.7	32	0	0–7.71	46	0	0–8.38	42	0	0–29.9	9
	2000/2001	0	0–7	51	0	0–6.21	58	0	0–8.97	39	0	0–11.7	29
	2001/2002	0	0–0.77	494	0	0–6.11	59	0	0–11.03	31	2.86	0.51–14.53	35
	2002/2003	0	0–2.01	187	0	0–6.21	58	0	0–6.53	55	0	0–8.2	43
	2003/2004	0	0–3.5	106	0	0–1.84	205	0	0–3.66	101	1.69	0.57–4.84	178
	2004/2005	0	0–0.47	828	0	0–1.41	268	0	0–2.45	153	1.33	0.45–3.85	225
	2005/2006	0	0–0.61	623	0	0–1.79	211	0	0–3.05	122	0	0–2.28	165
	2006/2007	0	0–0.85	448	0	0–2.36	159	0	0–4.69	78	0	0–4.32	85
	2007/2008	0	0–0.97	393	0	0–3.21	116	0	0–13.8	24	0.84	0.15–4.61	119
	2008/2009	0	0–1	380	0	0–2.63	142	0	0–8.97	39	1.31	0.36–4.64	153
	2009/2010	0	0–0.78	488	1.79	0.32–9.5	56	0	0–12.1	28	0	0–5.92	61

[a] Sampling year refers to period from July to June split over two calendar years.
[b] Outside (Outside of Riding Mountain Eradication Area [RMEA]).
[c] White-tailed deer.

TABLE 2: Summary of gross pathological and culture results for infected deer and elk by number of tissues examined from south-western Manitoba.

Species	Tissues Examined	No. Examined (%)	No. Cultured	M. bovis No.	%	M. avium No.	%	M. kansasii No.	%	M. terrae No.	%	Other Mycobacteria[a] No.	%
	Whole carcass	446 (12.3%)	445	31	6.97	5	1.12	0	0.00	4	0.90	2	0.45
	Head & Lungs[b]	2589 (71.5%)	2567	9	0.35	5	0.19	0	0.00	6	0.23	0	0.00
Elk	Head Only	571 (15.8%)	569	1	0.18	1	0.18	0	0.00	0	0.00	0	0.00
	Lungs Only[b]	14 (0.4%)	9	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	Total	3620	3590	41	1.17	11	0.31	0	0.00	10	0.28	2	0.06
	Whole carcass	452 (6.5%)	447	5	1.12	1	0.22	0	0.00	6	1.34	0	0.00
	Head & Lungs[b]	5198 (75.2%)	5176	5	0.10	2	0.04	1	0.02	12	0.23	0	0.00
WTD	Head Only	1208 (17.5%)	1192	1	0.08	1	0.08	0	0.00	0	0.00	0	0.00
	Lungs Only[b]	51 (0.7%)	47	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	Total	6909	6815	11	0.16	4	0.06	1	0.01	18	0.26	0	0.00

[a] One isolate was *M. chelonae* and one was *M. fortuitum*.
[b] Lung tissue including tracheobronchial and mediastinal lymphoid tissues.

TABLE 3: Site of gross visible lesions (GVL) in *M. bovis* positive elk and white-tailed deer from south-western Manitoba.

		Medial retropharyngeal lymph node	Parotid lymph node	Mandibular lymph node	Palatine tonsil	Lateral retropharyngeal lymph node	Lungs[a]	Body lymph nodes[b]	Abdominal lymph nodes[c]	No visible lesions
	GVL	12	9	2	19	2	20	10	7	0
Elk	Total tested	41	41	41	41	41	40	36	36	41
	Proportion	29.3	22.0	4.9	46.3	4.9	50.0	27.8	19.4	0.0
	GVL	8	1	0	0	0	2	1	2	0
WTD	Total tested	11	11	11	11	11	10	5	5	11
	Proportion	72.7	9.1	0.0	0.0	0.0	20.0	20.0	40.0	0.0

[a] Includes tracheobronchial and mediastinal lymphoid tissues.
[b] Includes prescapular, prefemoral, supramammary/testicular, and popliteal lymph nodes.
[c] Includes mesenteric, hepatic, portal, and internal iliac lymph nodes.

in and around RMNP during the period of this survey (Figure 2). Mean period prevalence over the twelve-year surveillance period was 0.89% (0.66%–1.21%) for elk and 0.15% (0.08%–0.27%) for white-tailed deer. A total of 41 culture positive elk and 11 culture positive white-tailed deer were detected through all forms of surveillance. Elk prevalence has varied quite dramatically from year to year with the highest prevalence being detected in the winter of 2002/2003 (2.01%, Figure 2) when 10 culture positive animals were found through blood testing within RMNP. Prevalence in white-tailed deer has been similarly low and consistently below 1% throughout this period. Virtually all infected elk and white-tailed deer have come from a small geographic area around the north-western border of RMNP (Table 1, Figure 1). This 1800 km^2 area designated the Western Control Zone where most management activities have been focussed, encompasses 37 of the 41 (90.2%) culture positive elk and 10 of 11 (90.9%) culture positive white-tailed deer found through all forms of surveillance since 1997. Annual prevalence of *M. bovis* within the Western Control Zone has been consistently higher than other

surveillance areas ranging from zero to 6.85% (Table 1). Elk from the WCZ were approximately 21.1 times more likely ($\chi^2 = 67.7$, $P < .001$) to be culture positive than elk from outside this area and white-tailed deer were approximately 49.1 times more likely ($\chi^2 = 56.4$, $P < .001$) to be culture positive compared to deer from outside this zone (based on pooled data from the other three zones for comparison). There was no evidence of a linear trend in overall prevalence for elk ($P = .827$), deer ($P = .80$) or both species combined ($P = .363$) when all data from 1997 to 2010 was examined. But if only the data from 2003 to 2010 was examined neither elk ($P = .120$) nor deer ($P = .768$) exhibit a linear trend, but both species combined exhibit a significant downward trend ($P = .019$) in this most recent time period, as can be observed in Figure 2. This time period also corresponds to a significant decline in number of elk and deer examined (Figure 2), although prevalence and sample numbers were not correlated ($\rho = -0.093$).

Mycobacterium bovis was the most common mycobacterial isolate cultured from elk, but *M. terrae* was the most frequent isolate from white-tailed deer (Table 2). *M. avium*

TABLE 4: Proportion of culture positive elk with gross visible lesions (GVL) in different tissues and body sections stratified by sex.

	Lung[a] GVL	Medial Retropharyngeal GVL	Parotid GVL	Tonsil GVL	Abdominal GVL[b]	Body GVL[c]
Male	13/20 (65.0)	6/22 (27.3)	7/22 (31.8)	11/22 (50.0)	4/15 (26.7)	5/15 (33.3)
Female	6/19 (31.6)	6/19 (31.6)	2/19 (10.5)	8/19 (42.1)	4/15 (26.7)	6/15 (40.0)
χ^2	4.24 (0.039)	0.089 (0.765)	2.63 (0.105)	0.249 (0.618)	0.0 (1.0)	0.139 (0.710)
Odds Ratio	4.02 (0.89–18.9)	0.81 (0.35–2.16)	3.97 (0.60–43.5)	1.38 (0.34–5.65)	1.0 (0.31–3.28)	0.75 (0.33–2.04)

[a] Includes tracheobronchial and mediastinal lymphoid tissues.
[b] Includes mesenteric, hepatic, portal, and internal iliac lymph nodes.
[c] Includes prescapular, prefemoral, supramammary/testicular, and popliteal lymph nodes.

TABLE 5: Prevalence of *M. bovis* in elk and white-tailed deer (WTD) stratified by sex, age category, and surveillance method from southwestern Manitoba.

Species	Age (Years)	Culture −	Culture +	Prevalence[a](%)	Odds Ratio	χ^2	P
	<1[b]	449	1	0.22	1		
	1 to 2	814	6	0.73	3.31 (0.40–152.6)	1.375	.241
	3–5	1821	11	0.60	2.71 (0.39–117.0)	0.987	.320
	6–8	508	12	2.31	10.61 (1.56–454)	7.93	.005
	>8	417	11	2.57	11.84 (1.7–510.9)	8.96	.003
Elk	Sex						
	Female[b]	2683	19	0.70	1		
	Male	1859	22	1.17	1.67 (0.86–3.27)	2.72	.099
	Surveillance Method						
	Hunted[b]	3345	9	0.27	1		
	Opportunistic	179	3	1.65	6.23 (1.07–25.2)	9.72	.002
	Culled	73	2	2.67	10.2 (1.05–50.3)	13.2	<.001
	Blood Test	945	27	2.78	10.6 (4.82–25.7)	57.5	<.001
	Age (Years)						
	<1	457	0	0.00	ND	ND	ND
	1–2[b]	2017	2	0.10	1		
	3–5	4476	3	0.07	0.68 (0.08–8.10)	0.186	.666
	6–8	220	6	2.65	27.5 (4.9–279.3)	37.4	<0.001
	>8	25	0	0.00	ND	ND	ND
	Sex						
WTD	Female	1976	1	0.05	1		
	Male	5392	10	0.19	3.66 (0.52–159.1)	1.76	.185
	Surveillance Method						
	Hunted	6735	6	0.09	1		
	Opportunistic	195	0	0.00	0 (0–29.5)	0.17	.677
	Culled	273	3	1.09	12.34 (1.98–58.1)	20.61	<.001
	Blood Test	165	2	1.20	13.61 (1.33–76.7)	17.31	.001

[a] Stratum specific prevalence (number positive/total number tested per category).
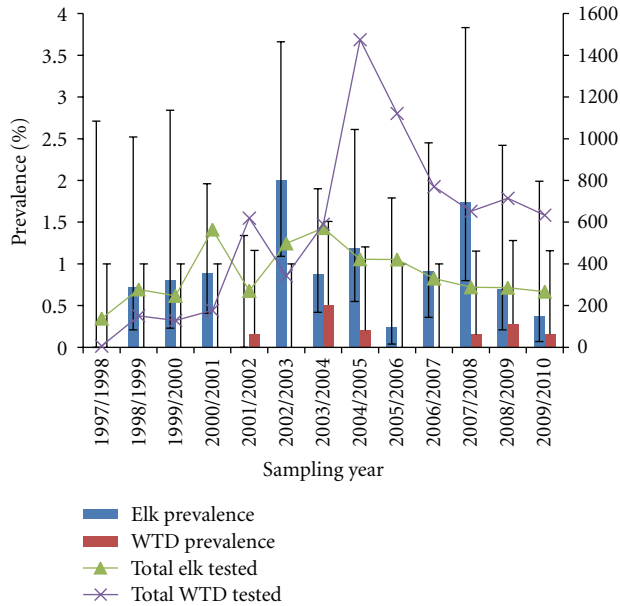[b] Category used as the reference category for odds ratio and chi-square calculations.

Figure 2: Overall estimated annual prevalence of *M. bovis* and total number of elk and white-tailed deer sampled from south-western Manitoba from 1997 to 2010.

was only cultured from elk, while *M. kansasii* was only cultured from deer. Other mycobacteria isolated included *M. fortuitum* and *M. chelonae*. All mycobacteria including *M. bovis* were most frequently isolated when the entire carcass was available for examination compared to other tissues such as the head or lungs. Thirty-one culture positive elk and 5 culture positive deer were diagnosed from examination and full necropsy of the entire carcass. Of these, 19 of 31 (61.3%) elk had gross visible lesions in the head, 15 of 31 (48.4%) had gross visible lesions in the lungs, and 25 of 31 (80.6%) had gross visible lesions in either the head lymph nodes or lungs. Three of 5 (60%) culture positive deer which had full necropsies had gross visible lesions in the head, 0 of 5 had gross visible lesions in the lungs, and 3 of 5 (60%) had gross visible lesions in either the head lymph nodes or lungs.

The most common sites of gross lesions in culture positive elk were the lungs, palatine tonsils, and retropharyngeal lymph nodes, while in white-tailed deer it was the retropharyngeal lymph node, abdomen (mesenteric lymph node), and body lymph nodes (popliteal) (Table 3). All (100%) culture positive white-tailed deer and elk exhibited at least one gross lesion compatible with *M. bovis* infection at necropsy. Gross lesions typically consisted of caseopurulent or granulomatous lesions which were either multifocal or singular and were commonly associated with some degree of mineralization. Histologically, lesions were typically well encapsulated when in lymphoid tissues and were often disseminated when in the lungs. Male elk were approximately four times more likely to have gross visible lesions in the lungs compared to female elk when stratified by sex (Table 4). Gross lesions did not vary significantly by sex for other tissues examined.

Neither sex was more likely to be *M. bovis* culture positive for both elk and deer based on proportions sampled in this study (Table 5). The prevalence of infection increased with age class in elk, but the oldest age class of deer (>8 years) had very few samples and no *M. bovis* positive animals. The majority of culture positive elk and deer were detected through blood testing, followed by opportunistic sampling and culling (Table 5), while fewer culture positive animals were detected with hunter-killed animals. At necropsy, blood-tested elk had odds of testing culture positive of 10.6 compared to hunter-killed elk, while blood-tested deer had odds of 13.6 compared to hunter-killed deer (Table 5). The seven culture positive elk in the younger age classes (less than or equal to 2 years of age) were all found prior to 2004, and no elk younger than five years of age has been found since then.

4. Discussion

Bovine tuberculosis has been consistently present in elk in the RMNP ecosystem since at least 1992 and in white-tailed deer sporadically since 2001. Culture positive elk have been found every year in this area with the exception of two years (1997/1998 and 2001/2002), while infected white-tailed deer have been detected only in certain years and not consistently from year to year despite testing large numbers of animals. For this reason, it has been suggested that elk are the primary reservoir species of *M. bovis* within this ecosystem [2]. The factors which result in this differential temporal occurrence could be related to differences in social structure, susceptibility to *M. bovis*, individual contact rate, herd immunity, and method of testing. Studies in Michigan and New Zealand suggest that elk or red deer do not act as reservoir species, but are spillover hosts instead [13, 27, 28] while data from red deer in France suggest they may act as a reservoir host in association with wild boar [27, 29]. Data presented in this study suggest that elk indeed may be a primary reservoir species, but that infected white-tailed deer may also be necessary to maintain ongoing infection in a multispecies reservoir system [9]. Infected cattle herds may also be a necessary part of this multispecies reservoir, as infected cattle herds have not been found consistently in this area despite rigorous and intensive testing [1, 3], but the role of cattle as a reservoir species is currently undetermined. It is unlikely that there are other undetected reservoir species in this ecosystem as multiple species have been assessed with negative findings to date [1, 3, 30]. This study provides some evidence that overall prevalence of *M. bovis* in both deer and elk is declining since 2003 as the number of infected cattle herds has also declined. Another piece of evidence which supports this is the lack of younger age classes of elk found positive since 2004. Since 2004, all *M. bovis* positive elk found through surveillance activities have been 5 years of age or older, but prior to 2004, five elk that were 2 years of age, 1 yearling and one calf were found to be infected. This trend is not apparent for white-tailed deer as the two most recent infected white-tailed deer were 2 years of age. Since *M. bovis* infection in cervids results in chronic disease and elk are a relatively long-lived ungulate species, especially

in a protected area, it is likely that positive cases of *M. bovis* will be continued to be detected in both elk and deer in this area for several years to come. The net force of infection is the instantaneous per capita rate that individual cervids become infected [31]. This can be estimated in wild populations infected with *M. bovis* using the proportion of young age classes found infected on cross-sectional surveys [13], as these represent relatively new infections based on short exposure times. Based on the findings of this study, the net force of infection has decreased in elk since 2004. Similar to previous studies of both red deer and white-tailed deer, age-specific prevalence of *M. bovis* increases dramatically in older age classes of both elk and deer [14, 16, 32]. Elk older than 6 years were 10 times more likely to be culture positive compared to younger age classes. Small numbers of positive deer made this association much less apparent with white-tailed deer, but the trend was similar.

The prevalence of *M. bovis* in wild elk is significantly lower in this ecosystem compared to comparable populations of red deer found in other parts of the world including New Zealand, Spain, and France where prevalence often exceeds 30%. Spatial aggregation at waterholes has been shown to be an important risk factor for infection in Spanish red deer [16], while association with other infected wildlife reservoirs such as brush-tailed possum and wild boar have been shown to be important risk factors in New Zealand and France, respectively [27, 29, 33]. The role of host density in maintenance of cervid reservoirs of *M. bovis* is somewhat equivocal with some studies finding density-dependent effects, while others have refuted this hypothesis [1, 15, 34]. Attempts to model *M. bovis* infection in wild ungulates have relied upon density-dependent transmission [35] and some studies have found positive correlations between density and prevalence [15]. Supplemental feeding and spatial aggregation around waterholes have been positively associated with spatial occurrence of *M. bovis* [10, 16], suggesting that contact structure and localized congregations may be important factors allowing maintenance and transmission of the disease in wildlife reservoirs. Elk densities were historically much higher in the RMNP area [36] and deer densities have likely been increasing since the early part of the twentieth century when white-tailed deer began colonizing this area. One of the management strategies instituted in 2003 to control *M. bovis* in this area was an attempt to keep the regional elk population at historically low levels in an attempt to reduce transmission [18]. Other strategies introduced at roughly the same time were lengthened hunting seasons, a moratorium on regional wolf trapping, and fencing of hay storage yards around RMNP [1, 37]. It appears that this combination of management factors has likely played a role in reducing the prevalence of *M. bovis* in ungulates in the RMNP area since 2003 as well as a decreasing the number of spillover events to surrounding cattle herds. Strategies to eventually eliminate bovine tuberculosis in this ecosystem are being actively considered by government agencies and local stakeholders.

The pathology of *M. bovis* infection found in elk is similar to that described in both captive and farmed elk as well as wild red deer populations in other parts of the world, with the exception that all culture positive elk had grossly visible lesions, meaning there were no culture positive elk without visible lesions (NVL) in this study. Other studies of wild red deer in Spain and New Zealand have found proportions of culture-positive elk that are NVL as high as 30% [7, 14], while studies in Canadian captive elk had proportions of approximately 7% [23]. The reason may be that a significant proportion of elk in this study were examined by a full necropsy using a detailed necropsy procedure that was designed to find *M. bovis* lesions, whereas other studies have typically used field necropsies or just examined portions of carcasses. Thus, many subtle lesions that may have been missed on a field necropsy were discovered during this study.

Other mycobacteria isolated from lesions in both elk and deer likely decrease the specificity of diagnostic tests for mycobacteria. *M. terrae* was the most common mycobacterial isolate in white-tailed deer, but previous studies have not reported isolation of *M. terrae* commonly [38]. *M. avium* was the next most common mycobacterial isolate in elk. Prior exposure to environmental mycobacteria such as *M. terrae* and other mycobacteria may play a role in sensitizing the host immune response to *M. bovis* [39, 40] and may be one factor causing individual heterogeneity in rates of infection and resistance in wild populations.

Both male elk and white-tailed deer were more likely to be culture positive for *M. bovis*, but the difference was not significant due to low sample sizes when stratified by species (Table 5). Males have generally had higher odds of testing positive to *M. bovis* in studies of both red deer and white-tailed deer [2, 32]. In the RMNP ecosystem, 10 of 11 culture-positive white-tailed deer have been male since 2001, but the low numbers of positives and higher proportion of male deer in the sample dilutes this effect. Sampling zone and surveillance method were significantly associated with *M. bovis* status in this study with animals being sampled in the Western Control zone being at a significantly higher risk of being positive for *M. bovis* than elk or deer sampled in other areas. Both elk and deer sampled through blood testing and culling were much more likely to be culture positive than animals sampled through hunting or other surveillance methods. One reason for this is that once *M. bovis* positive elk were found in the Western Control zone through blood sampling, surveillance efforts tended to focus on this area to a certain degree, increasing the likelihood of finding culture positive animals. Hunter samples tended to be more randomly distributed but are limited spatially in that none came from within RMNP. The true extent of *M. bovis* infection in this ecosystem was not fully realized until a costly and rigorous sampling program was carried out using blood tests within RMNP. Using multiple surveillance methods rather than relying on a single method was a key determinant in determining the extent of infection in wildlife in this ecosystem. Detection of *M. bovis* in wildlife species at fine spatial scales within protected areas is much more difficult [17], and this is one of the first studies to rely on blood sampling rather than traditional skin testing and hunter surveillance to determine *M. bovis* distribution in a cervid reservoir.

Similar to Michigan, *M. bovis* appears to be highly clustered in cervids in the RMNP area, but unlike Michigan,

elk are more commonly infected than white-tailed deer [8]. Reasons for this discrepancy are unknown, but are likely related to different population densities, social behaviour, and presence of baiting and feeding for hunting [10]. White-tailed deer densities in Michigan are much higher compared to south-western Manitoba [1] and the role of supplemental feeding to bait deer in Michigan [11] may act to further aggregate deer at local spatial scales. Supplemental feeding and baiting for purposes of hunting have been prohibited through legislation and enforced in the RMEA since 2002. Baiting and feeding is difficult to control in some jurisdictions, but has been relatively well accepted by local stakeholders in Manitoba. Conversely, elk population size and density are likely greater within RMNP than is found in Michigan, where elk densities are somewhat lower and not directly within the core area where *M. bovis* is found. Other factors such as habitat quality and quantity, intraspecific and interspecific contact rates, and herd immunity may also play a role in the maintenance of *M. bovis* infection in these wildlife reservoirs. Studies currently ongoing in the RMNP area hope to clarify the role of some of these important factors.

5. Conclusions

M. bovis infection has been consistently present in a relatively small geographic area located in and around the north-western part of RMNP since at least 1978, but significant annual variation in prevalence has occurred since 1997 in both elk and deer. Period prevalence in elk is approximately six times higher than deer, suggesting they may be a significant reservoir host of *M. bovis* in this ecosystem, but that infected white-tailed deer may also be required to maintain a true reservoir in this system. Pathological lesions associated with *M. bovis* infection and distribution of those lesions in wild elk and deer are very similar to those described in other parts of the world, but fewer NVL elk were found compared to red deer. The lack of culture positive animals in younger age classes of elk since 2003 indicate that the net force of infection as well as overall prevalence are declining in elk in this area, but further surveillance and monitoring will be necessary to determine if this is consistent over time. This study demonstrates that it is vitally important to sample all geographical sites occupied by *M. bovis* host species using a variety of surveillance methods if possible, or focal aggregations of disease may be overlooked for long periods of time. Both the management and surveillance of infected wildlife reservoirs is challenging and difficult, but blood-based assays were a crucial part of estimating the apparent prevalence and spatial distribution of *M. bovis* infection in this system.

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