DOI: 10.7589/JWD-D-20-00198

Journal of Wildlife Diseases, 57(3), 2021, pp. 683–688

Prevalence of *Mycoplasma* spp. in the Respiratory Tract of Healthy North American Bison (*Bison bison*) and Comparison with Serum Antibody Status

Karen B. Register,[1,10] Lee C. Jones,[2] William D. Boatwright,[1] Todd K. Shury,[3] Murray Woodbury,[4] Robert G. Hamilton,[5] John Treanor,[6] Neil Dyer,[7] and Pauline Nol[8,9] [1]Ruminant Diseases and Immunology Research Unit, USDA/Agricultural Research Service/National Animal Disease Center, 1920 Dayton Ave., Ames, Iowa 50010, USA; [2]US Fish and Wildlife Service, Wildlife Health Office, 10 E Babcock, Bozeman, Montana 59715, USA; [3]Parks Canada Agency, Western College of Veterinary Medicine, Department of Veterinary Pathology, 52 Campus Dr., Saskatoon, Saskatchewan S7N 5B4, Canada; [4]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Dr., Saskatoon, Saskatchewan S7N 5B4, Canada; [5]The Nature Conservancy, PO Box 458, Pawhuska, Oklahoma 74056, USA; [6]Yellowstone Center for Resources, PO Box 168, Yellowstone National Park, Wyoming 82190, USA; [7]North Dakota State University, Dept. 4000, PO Box 6050, Fargo, North Dakota 58108, USA; [8]Wildlife Livestock Disease Investigations Team, USDA:APHIS:Veterinary Services, 4101 LaPorte Ave., Fort Collins, Colorado 80521, USA; [9]Current address: Colorado Division of Parks and Wildlife, Wildlife Health Program, 4330 Laporte Ave., Fort Collins, Colorado 80521, USA; [10]Corresponding author (email: tekabeau@q.com, karen.register@usda.gov)

ABSTRACT: *Mycoplasma bovis* is a primary cause of respiratory and reproductive diseases in North American bison (*Bison bison*), with significant morbidity and mortality. The epidemiology of *M. bovis* in bison is poorly understood, hindering efforts to develop effective control measures. Our study considered whether healthy bison might be carriers of *M. bovis*, potentially serving as unrecognized sources of exposure. We used culture and PCR to identify mycoplasmas in the nasal cavity or tonsil of 499 healthy bison from 13 herds and two abattoirs in the US and Canada. *Mycobacterium bovis* was detected in 15 bison (3.0%) representing two herds in the US and one in Canada, while *M. bovirhinis*, *M. bovoculi*, *M. arginini*, or *M. dispar* was identified from an additional 155 bison (31.1%). *Mycoplasma bovirhinis* was identified most frequently, in 142 bison (28.5%) representing at least 10 herds. Of the 381 bison for which serum was available, only 6/13 positive for *M. bovis* (46.2%) tested positively with an *M. bovis* ELISA, as did 19/368 negative for *M. bovis* (5.2%). Our data reveal that *M. bovis* can be carried in the upper respiratory tract of healthy bison with no prior history or clinical signs of mycoplasmosis and that a large proportion of carriers may not produce detectable antibodies. Whether carriage of other mycoplasmas can trigger cross-reactive antibodies that may confound *M. bovis* serology requires further study.

Key words: Bison, ELISA, *Mycoplasma bovirhinis*, *Mycoplasma bovis*.

Mycoplasma bovis is a common cause of pneumonia, arthritis, and mastitis in cattle and is occasionally implicated in reproductive disorders (Maunsell et al. 2011; Ridley and Hately 2018). It imposes a considerable economic burden on cattle production worldwide (Dudek et al. 2020). Around the turn of this century *M. bovis* was recognized for the first time as a pathogen in North American bison (*Bison bison*; US Department of Agriculture 2013). Investigation of disease outbreaks revealed it as a primary cause of pneumonia, polyarthritis, necrotic pharyngitis, pleuritis, dystocia, and abortion, with high morbidity and significant mortality (Dyer et al. 2008; Janardhan et al. 2010; Dyer et al. 2013; Register et al. 2013b; Bras et al. 2016; Bras et al. 2017a). *Mycoplasma bovirhinis*, regarded as a commensal, is also frequently recovered from the respiratory tract of both healthy and diseased cattle (Maunsell and Donovan 2009), but whether it inhabits bison is unknown.

Efforts to control mycoplasmosis in cattle have been only marginally successful. Antibiotic treatment is frequently ineffective (Maunsell and Donovan 2009), and similarly poor outcomes are the norm in affected bison (US Department of Agriculture 2013). Cattle vaccines stimulate measurable immune responses but offer limited efficacy under field conditions (Maunsell and Donovan 2009) and are not recommended for use in bison. An autogenous vaccine containing bison isolates from outbreaks of mycoplasmosis is available in the US, but no data exist regarding its efficacy. Strategies to control the frequency and impact of mycoplasmosis in bison are urgently needed; their development is hampered by gaps in our

understanding of *M. bovis* epidemiology. One of several key unanswered questions is whether healthy bison harbor *M. bovis* in the absence of clinical signs, thereby serving as hidden sources of transmission. Studies in which apparently healthy bison with no history of mycoplasmosis tested positively for antibodies reactive with *M. bovis* are consistent with inapparent infection (Register et al. 2021; Bras et al. 2017b), but there are no definitive data based on the results of samples cultured from healthy bison. We aimed to determine whether *M. bovis* can be recovered from the respiratory tract of healthy bison. Sera from the same bison were tested with an *M. bovis* enzyme-linked immunosorbent assay (ELISA), and the results were compared with those from culture.

During 2012–19, we collected deep nasal swabs from 391 bison, each sampled only once, at 11 locations in the US (n=304) and two locations in Canada (n=87; see Table 1). Bison at 10 locations were wide-ranging and minimally managed, handled yearly or biennially (n=333); those at the other three sites were restrained by fencing and handled more frequently (n=58). Most bison (304/391; 77.7%) were adults when sampled; 21.5% (84/391) and 0.8% (3/391) were juveniles or calves, respectively. All bison appeared healthy at the time of sample collection; none had been previously vaccinated against *M. bovis*. Twelve herds had no prior history of mycoplasmosis. Herd USA-8 had suffered a clinical outbreak of *M. bovis* approximately 6 mo prior to the date of sample collection but not more recently. Tonsil swabs from healthy bison processed at abattoirs in the US (n=55) and Canada (n=53) were also evaluated.

Swabs were used to inoculate selective PPLO broth (PPLO broth supplemented with 0.05% thallium acetate and 500 IU/ml penicillin G). Cultures were incubated at 37 C in 5% CO_2 for at least 24 h, or until growth was visually apparent, up to 72 h in the event of no detectable growth. At the end of the incubation period 5 μL of every culture was tested in an *M. bovis*–specific PCR (Clothier et al. 2010). To identify other mycoplasmas potentially present in *M. bovis*–negative cultures, an additional 5 μL was tested with a PCR amplifying ~780-base pair of the 16S rRNA gene from *Mycoplasma* spp. known to infect cattle (Miles et al. 2004). Amplicons from positive reactions were treated with ExoSAP-IT (Thermofisher, Waltham, Massachusetts, USA) and sequenced at the Iowa State University DNA Facility (Ames, Iowa, USA). Consensus sequences were deduced from a minimum of two high-quality reads, with at least one from each strand. An aliquot of each first-passage culture was serially diluted and plated on selective PPLO agar. Plates were incubated until *Mycoplasma*-like colonies were evident, or up to 5 d in the case of negative cultures. Well-isolated, suspect colonies from PPLO plates were used to inoculate fresh PPLO broth, which was incubated and tested using the *M. bovis*–specific and 16S rRNA gene PCRs, as described earlier. Samples were considered positive on the basis of PCR results obtained with the first-passage broth culture, whether or not colonies were recovered from the subsequent passage on PPLO agar.

We collected lung tissue postmortem from 25 bison being culled from herd USA-2 at the time of sampling. All lungs appeared normal. Tissues were weighed, minced with sterile scissors, diluted 1:10 (weight/volume) in PPLO broth, and homogenized in C tubes using a gentleMACS Octo Dissociator (Miltenyi Biotec, Auburn, California, USA). For each homogenate, 300 μL was cultured in selective PPLO broth and tested for *M. bovis* and other mycoplasmas as described for nasal swabs.

We collected serum at the time that nasal swabs were obtained for 381 bison. Each was tested twice with the *M. bovis* BIO K 260 ELISA (Bio-X Diagnostics, Rochefort, Belgium) using independently made dilutions, as described previously (Register et al. 2013a). This ELISA has not been validated for use with bison but performed well in a prior study with bison with known exposure history to *M. bovis* (Register et al. 2013a). Mean test values were used to assign each sample a numeric score, from 0 (negative) to 5+, using the metric provided by the manufacturer. Sera were recorded as positive only when a result

TABLE 1. Year and age of 391 bison (*Bison bison*) in the USA (*n*=304) or Canada (*n*=87) from which nasal swabs (*n*=391) and sera (*n*=381) were collected between 2012 and 2019, size, geographic origin, and management status of the source herds, outcome of *Mycoplasma* culture of nasal swabs, and results from the BIO K 260 *M. bovis* enzyme-linked immunosorbent assay (ELISA).

Herd	Age of bison sampled (no.)	No. bison in herd	Geographic origin	Year	Management status[a]	No. with the indicated culture outcome/No. ELISA-positive						No. samples	
						Negative	*M. bovis*	*M. bovirhinis*	*M. bovoculi*	*M. arginini*	*M. dispar*	Swabs	Serum
USA-1	Adult (8)	~35	Iowa, USA	2012	RF		7/1	1/0				8	8
USA-1	Adult (31)	~35	Iowa, USA	2013	RF	18/1		13/1				31	31
USA-2	Adult (5), juvenile (6)	~89	Colorado, USA	2014	WR	4/0		7/0				11	11
USA-3	Adult (7), juvenile (11)	~55	Iowa, USA	2014	WR	15/0		3/0				18	18
USA-4	Adult (20), juvenile (13)	~570	Oklahoma, USA	2014	WR	30/3[b]		3/0[c]				33	30
USA-5	Adult (33)	~350	Nebraska, USA	2014	WR	20/0		5/0	7/0	1/0		33	33
USA-6	Adult (15), juvenile (11)	~335	Montana, USA	2014	WR	11/1		14/1	1/0			26	26
USA-7	Adult (8)	NA[d]	New Jersey, USA	2014	RF	8/0						8	8
USA-8	Adult (40)	~2100	Oklahoma, USA	2014	WR	21/4	1/1	18/1				40	40
USA-9	Adult (1), juvenile (4), calf (1)	~25	North Dakota, USA	2015	WR	6/0						6	6
USA-2	Adult (8), juvenile (12), calf (2)	~76	Colorado, USA	2015	WR	13/0		9/0				22	22
USA-10	Adult (1)	~10	New Jersey, USA	2015	RF	1/0[b]						1	0
Can-1	Adult (34), juvenile (1)	~500	Alberta, Canada	2017	WR	23/1[b]	4/3[b]	8/0				35	33
USA-1	Adult (10)	~30	Iowa, USA	2018	RF	3/0	2/1	2/1	3/1			10	10
USA-2	Adult (5), juvenile (4)	~180	Colorado, USA	2019	WR	7/0		2/1				9	9
USA-3	Juvenile (9)	~55	Iowa, USA	2019	WR	1/0		7/0			1/1	9	9
USA-11	Adult (37), juvenile (2)	~4585	Wyoming and Montana, USA	2019	WR	31/0		8/0				39	39
Can-2	Adult (41), juvenile (11)	~450	Saskatchewan, Canada	2019	WR	32/1[e]		20/1				52	48
Total						244/11	14/6	120/6	11/1	1/0	1/1	391	381

[a] RF = restrained by fencing, handled on multiple occasions per year; WR = wide-ranging, handled once yearly or biennially.

[b] Serum not available for one bison.

[c] Serum not available for two bison.

[d] Not available, owner declined to provide the information.

[e] Serum not available for four bison.

of 1+ or higher was obtained on both occasions tested. Results for 284 sera have been reported previously (Register et al. 2021).

Overall, 14/391 nasal swabs from bison (3.6%) were positive for *M. bovis*, with viable colonies recovered from each sample. Positive animals were identified in two herds in the US and one in Canada. Herd USA-1 accounted for 9/14 positives (64.3%), with positive bison found on two of the three occasions sampled. One positive bison was detected in herd USA-8, which had experienced an outbreak of mycoplasmosis 6 mo earlier. The remaining four positive bison were from herd Can-1. Herd-specific prevalence varied from 2.5% for USA-8 to 87.5% for USA-1 (in 2012).

First-passage nasal swab cultures from 133 *M. bovis*–negative bison tested positive with the *Mycoplasma*-specific 16S rRNA PCR (Table 1). The species in each positive culture was discerned based on the highest-scoring match obtained when the PCR amplicon sequence was used to query the National Center for Biotechnology Information nonredundant nucleotide database (2020). All top matches were ≥98% identical to ≥98% of the query sequence, with E values of 0.0. *Mycoplasma bovirhinis* was identified in 120 bison (30.7% of all bison), and *Mycoplasma*-like colonies identified as *M. bovirhinis* were recovered from all but 22 of the corresponding first-passage cultures. *Mycoplasma bovirhinis* was found in 10/13 herds or locations; the three remaining cannot confidently be considered free of *M. bovirhinis* since only a few individuals from each were tested (1–8 bison). Herd-level prevalence among those positive for *M. bovirhinis* ranged from 9.1% (USA-4) to 77.8% (USA-3, in 2019), mean 34.0%.

First-passage cultures from 11 bison representing three herds in the US were positive for *Mycoplasma bovoculi*, with colonies recovered from all but one. One bison from USA-5 was positive for *Mycoplasma arginini*; colonies of that organism were also obtained from that animal. One sample from USA-3 was positive for *Mycoplasma dispar*, but no colonies were recovered.

TABLE 2. Year and number of bison (*Bison bison*) culled from herd USA-2 (located in Colorado) between 2014 and 2019 from which nasal swabs, lung tissue, and sera were collected, outcome of *Mycoplasma* culture of nasal swabs and lungs, and results of the BIO K 260 *M. bovis* enzyme-linked immunosorbent assay (ELISA).

Year and no. bison	Culture outcome		ELISA
	Nasal cavity	Lung	
2014			
4	Negative	Negative	Negative
7	*M. bovirhinis*	Negative	Negative
2015			
5	*M. bovirhinis*	Negative	Negative
2019			
5	Negative	Negative	Negative
1	*M. bovirhinis*	*M. bovirhinis*	Negative
1	*M. bovirhinis*	*M. bovirhinis*	2+
2	Negative	*M. bovirhinis*	Negative

Only one abattoir-sampled bison tonsil, from the US, tested positively for *M. bovis* (0.9%); an isolate was obtained from that sample. Ten tonsils from the US and 12 from Canada were positive for *M. bovirhinis* (20.4%), with isolates recovered from 16.

Considering culture results from both nasal swabs (n=391) and tonsils (n=108), *M. bovis* was isolated from the upper respiratory tract of 15/499 bison (3.0%), while *M. bovirhinis* was detected in 142 (28.5%).

Mycoplasma bovirhinis was detected in first-passage cultures of four lung samples (16.0%), and *M. bovirhinis* colonies were recovered from two of those. Additionally, *M. bovirhinis* was detected in the nasal cavities of two bison with *M. bovirhinis*–positive lungs; no mycoplasmas were identified in the nasal cavities of the remaining two. No other *Mycoplasma* spp. were found in any other lung sample. Findings are summarized in Table 2, with results from nasal swabs and ELISA (described soon) included for comparison.

Antibodies reactive with *M. bovis* were detected in 25/381 bison sera tested (6.6%) but in only 46.2% of bison from which the mycoplasma was recovered (Table 3). Positive results were also obtained for 5.4% and 9.1%

TABLE 3. Summary of *Mycoplasma* culture results and BIO K 260 *M. bovis* enzyme-linked immunosorbent assay (ELISA) scores for 381 bison (*Bison bison*) in the USA (n=300) or Canada (n=81) from which nasal swabs and sera were obtained between 2012 and 2019.

Culture results	No. with ELISA score indicated				% ELISA positive
	0	1+	2+/3+	4+/5+	
M. bovis (n=14)[a]	7	2	3	1	46.2
M. bovirhinis (n=120)[b]	112	1	2	3	5.4
M. bovoculi (n=11)	10	0	0	1	9.1
M. arginini (n=1)	1	0	0	0	0
M. dispar (n=1)	0	1	0	0	100
Negative (n=244)[c]	226	4	5	2	4.6
Total	356	8	10	7	

[a] Serum not available for one bison.
[b] Serum not available for two bison.
[c] Serum not available for seven bison.

of bison culture-positive for *M. bovirhinis* or *M. bovoculi*, respectively, and 4.6% of bison in which no mycoplasmas were detected.

Our data reveal that *M. bovis* may reside in the upper respiratory tract of healthy bison with no apparent history or clinical signs of mycoplasmosis and that carriers may not produce detectable antibodies. Accordingly, the absence of neither clinical signs nor *M. bovis*–reactive antibody provides definitive evidence of freedom from infection with *M. bovis*. Whether healthy carriers are at higher risk for subsequent disease requires further investigation.

Our finding that 5.2% of *M. bovis*–negative bison were seropositive (19/368; Table 3), most with moderate to high levels of antibody (a score ≥2+), suggests possible cross-reactivity between antibodies elicited by other mycoplasmas and the ELISA capture antigen. Similarly, Bras et al. (2017b) found that 23/552 bison (4.2%) with no history of mycoplasmosis tested positively with a custom ELISA. We cannot dismiss the possibility that some bison *M. bovis*–negative at the time of sampling were seropositive owing to a prior, unrecognized infection. Also unknown is the limit of detection for *M. bovis* with our methods. Nonetheless, these explanations seem unlikely to fully account for our results, especially as we cultured other mycoplasmas more difficult to propagate than *M. bovis*. Studies directly addressing the question of cross-reactivity, including comparative immunoblotting with extracts from the mycoplasmas identified here as most prevalent among bison, are ongoing.

This study was conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the National Animal Disease Center and other participating institutions. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an Equal Opportunity Employer.

LITERATURE CITED

Bras AL, Barkema HW, Woodbury M, Ribble C, Perez-Casal J, Windeyer MC. 2016. Risk factors for *Mycoplasma bovis*-associated disease in farmed bison (*Bison bison*) herds in western Canada: A case-control study. *Prev Vet Med* 129:67–73.

Bras AL, Barkema HW, Woodbury MR, Ribble CS, Perez-Casal J, Windeyer MC. 2017a. Clinical presentation, prevalence, and risk factors associated with *Mycoplasma bovis*-associated disease in farmed bison (*Bison bison*) herds in western Canada. *J Am Vet Med Assoc* 250:1167–1175.

Bras AL, Suleman M, Woodbury M, Register K, Barkema HW, Perez-Casal J, Windeyer MC. 2017b. A serologic survey of *Mycoplasma* spp. in farmed bison (*Bison bison*) herds in western Canada. *J Vet Diagn Invest* 29:513–521.

Clothier KA, Jordan DM, Thompson CJ, Kinyon JM, Frana TS, Strait EL. 2010. *Mycoplasma bovis* real-time polymerase chain reaction assay validation and diagnostic performance. *J Vet Diagn Invest* 22:956–960.

Dudek K, Nicholas RAJ, Szacawa E, Bednarek D. 2020. *Mycoplasma bovis* infections—Occurrence, diagnosis and control. *Pathogens* 9:640.

Dyer N, Hansen-Lardy L, Krogh D, Schaan L, Schamber E. 2008. An outbreak of chronic pneumonia and polyarthritis syndrome caused by *Mycoplasma bovis* in feedlot bison (*Bison bison*). *J Vet Diagn Invest* 20: 369–371.

Dyer N, Register KB, Miskimins D, Newell T. 2013. Necrotic pharyngitis associated with *Mycoplasma bovis* infections in American bison (*Bison bison*). *J Vet Diagn Invest* 25:301–303.

Janardhan KS, Hays M, Dyer N, Oberst RD, Debey BM. 2010. *Mycoplasma bovis* outbreak in a herd of North American bison (*Bison bison*). *J Vet Diagn Invest* 22: 797–801.

Maunsell FP, Donovan GA. 2009. *Mycoplasma bovis* infections in young calves. *Vet Clin North Am Food Anim Pract* 25:139–177.

Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, Janzen ED. 2011. *Mycoplasma bovis* infections in cattle. *J Vet Intern Med* 25:772–783.

Miles K, McAuliffe L, Ayling RD, Nicholas RA. 2004. Rapid detection of *Mycoplasma dispar* and *M. bovirhinis* using allele specific polymerase chain reaction protocols. *FEMS Microbiol Lett* 241:103–107.

National Center for Biotechnology Information. 2020. *Basic local alignment search tool*. http://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed February 2021.

Register KB, Parker M, Patyk KA, Sweeney SJ, Boatwright WD, Jones LC, Woodbury M, Hunter DL, Treanor J, Kohr M, et al. 2021. Serological evidence for historical and present-day exposure of North American bison to *Mycoplasma bovis*. *BMC Vet Res* 17:18.

Register KB, Sacco RE, Olsen SC. 2013a. Evaluation of enzyme-linked immunosorbent assays for detection of *Mycoplasma bovis*-specific antibody in bison sera. *Clin Vaccine Immunol* 20:1405–1409.

Register KB, Woodbury MR, Davies JL, Trujillo JD, Perez-Casal J, Burrage PH, Clark EG, Windeyer MC. 2013b. Systemic mycoplasmosis with dystocia and abortion in a North American bison (*Bison bison*) herd. *J Vet Diagn Invest* 25:541–545.

Ridley A, Hately G. 2018. *Mycoplasma bovis* investigations in cattle. *Vet Rec* 183:256–258.

US Department of Agriculture. 2013. *Mycoplasma bovis*—An emerging pathogen in ranched bison. *Document 222.0913*. USDA:APHIS:VS:Center for Epidemiology and Animal Health, Fort Collins, CO, 6 pp.

Submitted for publication 12 November 2020.
Accepted 23 February 2021.