DOI: 10.7589/2011-09-261 *Journal of Wildlife Diseases*, 48(4), 2012, pp. 918–924

DETECTION OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN SEVERAL HERDS OF ARCTIC CARIBOU (*RANGIFER TARANDUS* SSP.)

Taya Forde,[1] Karin Orsel,[1] Jeroen De Buck,[1] Steeve D. Côté,[2] Christine Cuyler,[3] Tracy Davison,[4] Brett Elkin,[5] Allicia Kelly,[6] Martin Kienzler,[7] Richard Popko,[8] Joëlle Taillon,[2] Alasdair Veitch,[8] and Susan Kutz[1,9]

[1] Faculty of Veterinary Medicine, University of Calgary, 3350 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada
[2] Département de Biologie et Centre d'études Nordiques, Université Laval, 1045 Avenue de la Médecine, Québec, Québec G1V 0A6, Canada
[3] Greenland Institute of Natural Resources, Kivioq 2, PO Box 570, 3900 Nuuk, Greenland
[4] Department of Environment and Natural Resources, Government of the Northwest Territories, Bag Service #1, Shell Lake, Inuvik, Northwest Territories X0E 0T0, Canada
[5] Department of Environment and Natural Resources, Government of the Northwest Territories, 500-5102 50th Avenue, Yellowknife, Northwest Territories X1A 3S8, Canada
[6] Department of Environment and Natural Resources, Government of the Northwest Territories, Box 900, Ft. Smith, Northwest Territories X0E 0P0, Canada
[7] Department of Environment, Government of Yukon, Box 600, Dawson City, Yukon Territory Y0B 1G0, Canada
[8] Department of Environment and Natural Resources, Government of the Northwest Territories, PO Box 130, Norman Wells, Northwest Territories X0E 0V0, Canada
[9] Corresponding author (email: skutz@ucalgary.ca)

ABSTRACT: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a common pathogen in domestic ruminants that causes granulomatous inflammation of the small intestine leading to emaciation and wasting. Clinical disease (Johne's disease) is also reported for several wild ruminant species. Between 2007 and 2009 we collected 561 fecal samples from caribou (*Rangifer tarandus* ssp.) representing 10 herds of migratory caribou, two herds of caribou from Greenland, and three populations of boreal woodland caribou. Feces were tested for MAP by bacterial culture and PCR targeting the IS*900* insertion sequence. In total, 31 samples from eight different populations representing all three ecotypes were found positive for MAP by PCR, with one sample from the Rivière-aux-Feuilles herd also being culture positive for the type II (cattle) strain. The proportion of positive animals was particularly high in the Akia-Maniitsoq herd in Greenland, and Rivière-aux-Feuilles and Rivière-George herds in northeastern Canada (23.4, 11.5, and 10.0%, respectively). Our results indicate that MAP is present in several caribou herds of different ecotypes in northern Canada and Greenland and that MAP circulates within wildlife populations that do not have ongoing contact with domestic livestock. The epidemiology, pathogenicity, and effects on the health of caribou in northern ecosystems remain unknown.

Key words: Arctic, caribou, epidemiology, Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis*, *Rangifer*.

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease, an economically important infectious enteropathy in domestic ruminants worldwide (Ott et al., 1999). Clinical disease associated with MAP infection, including protein loss, dehydration, wasting, and inevitably death, is also reported in bighorn sheep (*Ovis canadensis*), bison (*Bison bison*), elk (*Cervus canadensis*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and reindeer (*Rangifer tarandus tarandus*; Poddoubski, 1957; Katic, 1961; Jessup et al., 1981; Williams et al., 1983; Buergelt et al., 2000; Balseiro et al., 2008; Clark et al., 2010; Forde et al., 2012). There is no treatment for this disease in any species (Collins, 2010). *Mycobacterium avium* subspecies *paratuberculosis* is primarily transmitted by the fecal–oral route, although intrauterine infection may also play a role (Whittington and Windsor, 2009). *Mycobacterium avium* subspecies *paratuberculosis* can survive for several months in manure at cool temperatures (Jorgensen, 1977) but cannot replicate outside of a host (Whittington and Sergeant, 2001).

Caribou and reindeer (*R. tarandus* spp.) are keystone species in the Arctic and Subarctic and central to the cultural, socioeconomic, and physical well-being of many circumarctic peoples. The health and sustainability of *Rangifer* is thus of high priority to circumpolar nations. Johne's disease described in semidomestic reindeer herds has an acute onset and results in a high mortality rate (Katic, 1961). In this species, diarrhea is generally absent and weight loss is the typical sign of disease (Poddoubski, 1957). *Mycobacterium avium* subspecies *paratuberculosis* antibody-positive semidomestic reindeer (Tryland et al., 2004) and wild caribou (Johnson et al., 2010) are reported, and MAP has been isolated from tissues of captive reindeer in poor body condition in Yukon, Canada (Merchant, pers. comm.). Our goal was to determine if MAP occurs in wild caribou in northern Canada and Greenland and establish baseline data regarding its distribution among these herds.

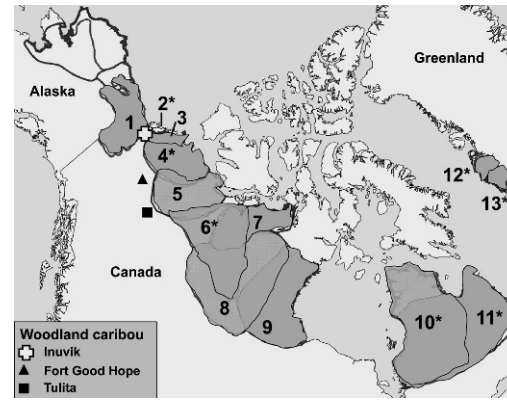

FIGURE 1. Caribou (*Rangifer tarandus* ssp.) tested for *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Herds sampled were 1) Porcupine; 2) Tuktoyaktuk Peninsula; 3) Cape Bathurst; 4) Bluenose-West; 5) Bluenose-East; 6) Bathurst; 7) Ahiak; 8) Beverly; 9) Qamanirjuaq; 10) Rivière-aux-Feuilles; 11) Rivière-George; 12) Kangerlussuaq-Sisimiut; and 13) Akia-Maniitsoq. Samples from boreal woodland caribou in the vicinity of the communities of Inuvik, Fort Good Hope, and Tulita, Northwest Territories, were also analyzed. Herds that had at least one MAP-positive fecal sample are indicated by an asterisk. One positive sample was also obtained from a woodland caribou in the Inuvik area.

MATERIALS AND METHODS

From 2007 to 2009, as an International Polar Year initiative, the CircumArctic Rangifer Monitoring and Assessment (CARMA) Network did extensive and intensive systematic health assessments on several circumarctic *Rangifer* herds (CARMA, 2011). Feces were sampled for MAP from 10 migratory herds in Canada, two herds from Greenland, and three populations of boreal woodland caribou, representing three different ecotypes (Festa-Bianchet et al., 2011; Fig. 1). Some herds were sampled on more than one occasion, and sampling occurred in the spring (March–June) or fall (September–October). Animals targeted for sampling varied depending on the monitoring objectives for each herd, and therefore individuals were not randomly selected. The majority of the samples were from harvested caribou, although some were through live capture-release. A minimum of 20 fecal pellets was collected from each individual and stored at −20 C until processing. The information gathered on individual caribou at the time of sampling varied, but when possible, the sex was reported and the animal's age estimated. Animals were assigned to one of three age classes: calves (less than 1 year of age), yearlings, and adults (animals 2 years of age and older). For some of the animals sampled, more precise age estimates were possible through cementum analysis of the first incisor (Miller, 1974).

Fecal decontamination, culture, and DNA extraction were performed as previously described (Forde et al., 2012). Briefly, samples were cultured in liquid medium using ESP para-JEM culture solution and incubated at 37 C in the ESP Culture System II Trek Machine (TREK Diagnostic Systems, Cleveland, Ohio, USA) for 8 weeks before extracting genomic DNA. A sample was considered culture positive if a sigmoidal curve was produced on the TREK machine, with confirmation by acid-fast staining, subculture on solid 7H11 agar supplemented with 2mg/L mycobactin J (Allied Monitor, Fayette, Missouri, USA), and IS*900* PCR.

The DNA extraction and PCR were performed on the culture broth from all samples after incubation. For samples received in early 2008, a nested PCR protocol was performed on extracted genomic DNA using the previously described L/AV primer set (Bull et al., 2003) for IS*900* amplification. Cycling conditions were as described by Bull et al.

(2003), except that 27 cycles were included for the first round, 35 for the second round, and the extension step was shortened to 2.5 min in both rounds. In early 2008 our laboratory was accredited through the US Department of Agriculture for the Johne's disease fecal culture proficiency test using a postculture confirmatory standard PCR protocol targeting IS*900*, which was more practical than the nested protocol for processing high numbers of samples. This protocol (Vary et al., 1990) with slight modifications (Forde et al., 2012) was used to test all samples received from summer 2008 onward.

The strain type of the culture-positive sample was determined by amplifying and sequencing the MAP1506 locus (University Core DNA Services, University of Calgary, Alberta, Canada). Amplification of this target was performed as previously described, and its sequence compared with the known single nucleotide polymorphism profile for types I, II, and III strains (Castellanos et al., 2010).

To determine whether there was a relationship between sex or age and the probability of testing positive for MAP, logistic regression was performed using herd, season, and year of sampling as fixed factors, sex or age class as explanatory variables, and MAP status (positive or negative) as the dependent outcome variable (IBM SPSS Statistics 19, IBM, Armonk, New York, USA). We did not investigate whether significant differences existed between herds due to the nonrandom nature of sampling.

RESULTS

Feces were tested from 114 males, 446 females, and one animal of unreported sex (Table 1). The age distribution was 19 calves, 23 yearlings, and 511 adults; the age category was not recorded for eight animals. The cementum age was available for 254 adults and ranged from 2 to 13 years with a median of 6 years.

A total of 31 caribou (5.5%) from eight different populations was positive by fecal PCR (Table 1). Only one of these, a yearling from the Rivière-aux-Feuilles herd, was culture positive and was identified as type II (cattle type) strain. The herds with the highest proportion of MAP-positive animals were Akia-Maniitsoq, Rivière-aux-Feuilles, and Rivière-George, with 23.4, 11.5, and 10.0% of samples testing positive, respectively. All but one positive sample were from females. Positive samples were found in all three age classes; three calves approximately 9–10 months old (15.8%), two yearlings (8.7%), and 26 adults (5.1%). Cementum age estimates were available for 22 of the 26 positive adults. The median age was 6.8 years (range 2–11.5 years). When corrected for herd, season, and year of sampling, there was no statistically significant association between either sex (B value=1.25, P=0.05) or age class (B value=0.67, P=0.24) and the MAP status of an individual.

DISCUSSION

In this first systematic survey for MAP in wild caribou across a wide geographic range, we detected MAP in more than half of the herds tested and in all three ecotypes, demonstrating a broad geographic distribution. There was a wide range in the proportion of positive samples among herds, with as high as 23.4% of samples positive in the Akia-Maniitsoq herd in Greenland. Strain typing of the culture-positive case from the Rivière-aux-Feuilles herd found the isolate to be a type II strain, the strain type that has the broadest host range and is most often isolated from wildlife (Stevenson et al., 2009). Further characterization will be necessary to determine whether this isolate differs significantly from other strains found in Canadian wildlife or livestock.

Although a much higher proportion of females than males was positive for MAP, this was not statistically significant after controlling for herd, season, and year. Herds that had the highest proportion of MAP-positive samples (Akia-Maniitsoq, Rivière-aux-Feuilles, and Rivière-George) exclusively sampled females. It is far more likely that there is an association between infection status and geographic location than infection status and the sex of the animal. Although there is no reason to believe that females are more susceptible to MAP or have a higher likelihood of shedding the bacterium, it has

TABLE 1. Caribou (*Rangifer tarandus* ssp.) herds sampled through the CircumArctic Rangifer Monitoring and Assessment (CARMA) Network and tested for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by fecal culture and PCR. The number of caribou tested between 2007 and 2009, the number of PCR-positive samples per herd, and the age class distribution of positive samples are presented. Herds are of the migratory ecotype unless otherwise stated.

Herd	Sample size	Number (percentage) of positive samples	Age class distribution of positive samples (calves/yearlings/adults)
Porcupine	23	0	-
Tuktoyaktuk Peninsula	28	1 (3.6)	0/0/1
Cape Bathurst	22	0	-
Bluenose-West	52	2 (3.8)	0/0/2
Bluenose-East	20	0	-
Bathurst	109	2 (1.8)	1/0/1
Beverly-Ahiak[a]	36	0	-
Beverly-Qamanirjuaq[a]	46	0	-
Rivière-aux-Feuilles	61	7 (11.5)[b]	0/1/6
Rivière-George	60	6 (10)	0/1/5
Kangerlussuaq-Sisimiut[c]	40	1 (2.5)	0/0/1
Akia-Maniitsoq[c]	47	11 (23.4)	2/0/9
Boreal Woodland Caribou[d]	17	1 (5.9)	0/0/1
Total	561	31 (5.5)	3/2/26

[a] Samples were collected from an area where there was herd overlap and individuals could not be designated to a specific herd of origin.

[b] One of these samples, from a female yearling, was also culture positive.

[c] Greenland ecotype.

[d] Boreal ecotype. Ten caribou sampled from Inuvik area (including one positive), two caribou sampled from Fort Good Hope area, and five caribou sampled from Tulita area.

been suggested that clinical disease in both cattle and reindeer may be precipitated by the stress of calving (Katic, 1961; Whittington and Sergeant, 2001).

It is interesting that we detected MAP in calves as young as 9–10 months old. This suggests that the pathogenesis of MAP infection in caribou could be similar to that seen in other cervids, which progress more quickly from silent to subclinical infection than domestic cattle or sheep (Manning et al., 1998). Bovine calves as young as 6 months of age and domestic sheep as young as 12 months can shed MAP, although it is exceptional for shedding to occur this early in these species (Whittington and Sergeant, 2001). The three positive calves in this survey were all from herds where at least one positive adult was also found.

It is uncertain whether the positive animals detected in this survey were truly infected with MAP. The phenomenon of "passive shedding" of MAP in feces, wherein an animal ingests MAP and subsequently sheds the bacterium in its feces without becoming infected, is possible, but is more likely in heavily contaminated environments (Pradhan et al., 2011). In our study, it is likely that positive fecal samples reflected true infections; however, this can only be confirmed through tissue culture or histopathology.

Clinical signs of Johne's disease can be subtle, with weight loss being the primary symptom in *Rangifer* (Poddoubski, 1957). Body-condition indices can vary substantially depending on season, age, and reproductive status (Chan-McLeod et al., 1995), and because of this variability and our small sample size, statistical analyses to investigate associations between body-condition parameters and MAP status were not pursued.

It is probable that our survey underestimates the true occurrence of MAP in these populations. Sample storage condi-

tions can affect MAP viability and the subsequent ability to culture (Khare et al., 2008). Some of our samples were frozen for up to 2 years before processing and may have experienced freeze–thaw cycles during transportation. This could have decreased our ability to culture MAP, particularly from low shedders. Although culture is considered the "gold standard" for MAP diagnosis (Whittington, 2010), higher sensitivity using fecal PCR is reported (Kawaji et al., 2007; Alinovi et al., 2009), and was the case in our study. Also influencing our ability to detect MAP is that animals may not begin to shed the bacterium in their feces until after a long incubation period, followed by a time of intermittent shedding. Finally, the number of bacteria shed by subclinically infected animals may be below the threshold of detection (Whittington and Sergeant, 2001).

Some of the variability in the proportion of positive samples between herds may be attributed to the change in our PCR technique. One hundred twenty-two samples were processed using nested PCR, including all the samples from the Akia-Maniitsoq herd. Nested PCR is a more sensitive technique, but is labor intensive and can be prone to false-positive results due to cross-contamination (Bölske and Herthnek, 2010). To confirm that the initial nested PCR results were true positives, a subset ($n=6$) of these positive fecal samples were re-extracted and again found to be positive.

Although we have demonstrated that MAP is present in wild caribou herds, it remains unknown how it circulates within these northern ecosystems. With few exceptions, for example, bison (*B. bison*) in northern Canada (Sibley et al., 2007) and key deer (*Odocoileus virginianus clavium*) in Florida (Quist et al., 2002), it is assumed that wildlife contract MAP from contact with domestic ruminants. In this survey, however, only in the Godthåb fjord region of west Greenland near the range of the Akia-Maniitsoq herd, where domestic sheep both historically and currently share parts of the caribou range (Cuyler, unpubl. data), has there been a potential for interaction and exchange of pathogens between wild caribou and domestic ruminants. *Mycobacterium avium* subspecies *paratuberculosis* is not known to replicate in the environment and therefore must be maintained within the caribou populations, in other ruminants, or possibly in other reservoir species, as has been previously suggested (Daniels et al., 2003). One mechanism for MAP circulation within caribou herds may be vertical transmission. A meta-analysis of in utero MAP infection in cows showed that approximately 9% of fetuses from subclinically infected cows are positive, and nearly 40% of clinically infected cows will give birth to infected offspring (Whittington and Windsor, 2009). In the future, MAP genotyping could be used to determine whether in utero infection in caribou is a potentially important route of transmission.

As the pathogenicity of MAP infection in caribou and the potential implications for herd health are unknown, monitoring of herd-level prevalence and strain typing of isolates from caribou would be valuable to gain insight into infection dynamics in this species. Performing tissue culture on individuals with positive fecal results for MAP would provide confirmation of true infection status as opposed to passive shedding. It is unlikely that MAP is a major driver for caribou population dynamics; however, in the presence of other stressors—including habitat fragmentation and climate change—infectious diseases and associated immunosuppression could become an increasingly important issue for the long-term health of wild caribou.

ACKNOWLEDGMENTS

We thank Amanda Reith, Dean Brown, and Jesse Invik for their hard work and dedication in processing the fecal samples, Jillian Steele for creating the map of the herds tested, and Julie Ducrocq and Ryan Brook for database management. A great many people, from community members to wildlife managers, were involved in sample collection through the CARMA net-

work, without whom a survey of this magnitude could not have been completed. Funding for this project was provided by the Canadian Federal International Polar Year Program, the Natural Sciences and Engineering Research Council of Canada, and Alberta Innovates.

LITERATURE CITED

Alinovi, C. A., M. P. Ward, T. L. Lin, G. E. Moore, and C. C. Wu. 2009. Real-time PCR, compared to liquid and solid culture media and ELISA, for the detection of *Mycobacterium avium* ssp. *paratuberculosis*. Veterinary Microbiology 136: 177–179.

Balseiro, A., J. F. García Marín, P. Solano, J. M. Garrido, and J. M. Prieto. 2008. Histopathological classification of lesions observed in natural cases of paratuberculosis in free-ranging fallow deer (*Dama dama*). Journal of Comparative Pathology 138: 180–188.

Bölske, G., and D. Herthnek. 2010. Diagnosis of paratuberculosis by PCR. *In* Paratuberculosis: Organism, disease, control, M. A. Behr and D. M. Collins (eds.). CAB International, Cambridge, Massachusetts, pp. 267–283.

Buergelt, C. D., A. W. Layton, P. E. Ginn, M. Taylor, J. M. King, P. L. Habecker, E. Mauldin, R. Whitlock, C. Rossiter, and M. T. Collins. 2000. The pathology of spontaneous paratuberculosis in the North American bison (*Bison bison*). Veterinary Pathology 37: 428–438.

Bull, T. J., E. J. McMinn, K. Sidi-Boumedine, A. Skull, D. Durkin, P. Neild, G. Rhodes, R. Pickup, and J. Hermon-Taylor. 2003. Detection and verification of *Mycobacterium avium* subsp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. Journal of Clinical Microbiology 41: 2915–2923.

CARMA. 2011. Mission statement, http://www.carmanetwork.com/pages/viewpage.action?pageId=1114187. Accessed June 2011.

Castellanos, E., A. Aranaz, and J. De Buck. 2010. Rapid identification and differentiation of *Mycobacterium avium* subspecies *paratuberculosis* types by use of real-time PCR and high-resolution melt analysis of the MAP1506 locus. Journal of Clinical Microbiology 48: 1474–1477.

Chan-McLeod, A. C. A., R. G. White, and D. E. Russell. 1995. Body mass and composition indices for female barren-ground caribou. Journal of Wildlife Management 59: 278–291.

Clark, R. G., J. F. T. Griffin, and C. G. Mackintosh. 2010. Johne's disease caused by *Mycobacterium avium* subsp. *paratuberculosis* infection in red deer (*Cervus elaphus*): An histopathological grading system, and comparison of paucibacillary and multibacillary disease. New Zealand Veterinary Journal 58: 90–97.

Collins, M. T. 2010. *Mycobacterium avium* subsp. *paratuberculosis* and antimicrobial agents. *In* Paratuberculosis: Organism, disease, control, M. A. Behr and D. M. Collins (eds.). CAB International, Cambridge, Massachusetts, pp. 138–143.

Daniels, M. J., M. R. Hutchings, P. M. Beard, D. Henderson, A. Greig, K. Stevenson, and J. M. Sharp. 2003. Do non-ruminant wildlife pose a risk of paratuberculosis to domestic livestock and vice versa in Scotland? Journal of Wildlife Diseases 39: 10–15.

Festa-Bianchet, M., J. C. Ray, S. Boutin, S. D. Côté, and A. Gunn. 2011. Conservation of caribou (*Rangifer tarandus*) in Canada: An uncertain future. Canadian Journal of Zoology 89: 419–434.

Forde, T., S. Kutz, J. De Buck, A. Warren, K. Ruckstuhl, M. Pybus, and K. Orsel. 2012. Occurrence, diagnosis and strain typing of *Mycobacterium avium* subspecies *paratuberculosis* infection in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in southwestern Alberta, Canada. Journal of Wildlife Diseases 48: 1–11.

Jessup, D. A., B. Abbas, and D. Behymer. 1981. Paratuberculosis in tule elk in California. Journal of the American Veterinary Medical Association 179: 1252–1254.

Johnson, D., N. J. Harms, N. C. Larter, B. T. Elkin, H. Tabel, and G. Wei. 2010. Serum biochemistry, serology, and parasitology of boreal caribou (*Rangifer tarandus caribou*) in the Northwest Territories, Canada. Journal of Wildlife Diseases 46: 1096–1107.

Jorgensen, J. B. 1977. Survival of *Mycobacterium paratuberculosis* in slurry. Nordisk Veterinaermedicin 29: 267–270.

Katic, I. 1961. Paratuberculosis with special reference to captive wild animals. Nordisk Veterinaermedicin 13: 205–214.

Kawaji, S., D. L. Taylor, Y. Mori, and R. J. Whittington. 2007. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in ovine faeces by direct quantitative PCR has similar or greater sensitivity compared to radiometric culture. Veterinary Microbiology 125: 36–48.

Khare, S., L. G. Adams, J. Osterstock, A. Roussel, and L. David. 2008. Effects of shipping and storage conditions of fecal samples on viability of *Mycobacterium paratuberculosis*. Journal of Clinical Microbiology 46: 1561–1562.

Manning, E. J. B., H. Steinberg, K. Rossow, G. R. Ruth, and M. T. Collins. 1998. Epizootic of paratuberculosis in farmed elk. Journal of the American Veterinary Medical Association 213: 1320–1322.

Miller, F. L 1974. Biology of the Kaminuriak population of barren-ground caribou. Part 2. Dentition as an indicator of sex and age; composition and socialization of the population.

Canadian Wildlife Service Report Series No. 31. 88 pp.

OTT, S. L., S. J. WELLS, AND B. A. WAGNER. 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Preventive Veterinary Medicine 40: 179–192.

PODDOUBSKI, I. V. 1957. La paratuberculose. Bulletin of the Office of International Epizootics 48: 469–476.

PRADHAN, A. K., R. M. MITCHELL, A. J. KRAMER, M. J. ZURAKOWSKI, T. L. FYOCK, R. H. WHITLOCK, J. M. SMITH, E. HOVINGH, J. S. VAN KESSEL, J. S. KARNS, AND Y. H. SCHUKKEN. 2011. Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* in a longitudinal study of three dairy herds. Journal of Clinical Microbiology 49: 893–901.

QUIST, C. F., V. F. NETTLES, E. J. B. MANNING, D. G. HALL, J. K. GAYDOS, T. J. WILMERS, AND R. R. LOPEZ. 2002. Paratuberculosis in key deer (*Odocoileus virginianus clavium*). Journal of Wildlife Diseases 38: 729–737.

SIBLEY, J. A., M. R. WOODBURY, G. D. APPLEYARD, AND B. ELKIN. 2007. *Mycobacterium avium* subspecies *paratuberculosis* in bison (*Bison bison*) from Northern Canada. Journal of Wildlife Diseases 43: 775–779.

STEVENSON, K., J. ALVAREZ, D. BAKKER, F. BIET, L. DE JUAN, S. DENHAM, Z. DIMARELI, K. DOHMANN, G. F. GERLACH, I. HERON, M. KOPECNA, L. MAY, I. PAVLIK, J. M. SHARP, V. C. THIBAULT, P. WILLEMSEN, R. N. ZADOKS, AND A. GREIG. 2009. Occurrence of *Mycobacterium avium* subspecies *paratuberculosis* across host species and European countries with evidence for transmission between wildlife and domestic ruminants. BMC Microbiology 9: 212.

TRYLAND, M., I. OLSEN, T. VIKØREN, K. HANDELAND, J. M. ARNEMO, J. THARALDSEN, B. DJØNNE, T. D. JOSEFSEN, AND L. J. REITAN. 2004. Serologic survey for antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in free-ranging cervids from Norway. Journal of Wildlife Diseases 40: 32–41.

VARY, P. H., P. R. ANDERSEN, E. GREEN, J. HERMON-TAYLOR, AND J. J. MCFADDEN. 1990. Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. Journal of Clinical Microbiology 28: 933–937.

WHITTINGTON, R. J. 2010. Cultivation of *Mycobacterium avium* subsp. *paratuberculosis*. *In* Paratuberculosis: Organism, disease, control, M. A. Behr and D. M. Collins (eds.). CAB International, Cambridge, Massachusetts, pp. 244–266.

WHITTINGTON, R. J., AND E. S. G. SERGEANT. 2001. Progress towards understanding the spread, detection and control of *Mycobacterium avium* subsp *paratuberculosis* in animal populations. Australian Veterinary Journal 79: 267–278.

———, AND P. A. WINDSOR. 2009. In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis. Veterinary Journal 179: 60–69.

WILLIAMS, E. S., S. P. SNYDER, AND K. L. MARTIN. 1983. Pathology of spontaneous and experimental infection of North American wild ruminants with *Mycobacterium paratuberculosis*. Veterinary Pathology 20: 274–290.

Submitted for publication 6 September 2011.
Accepted 13 June 2012.