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FEASIBILITY OF USING COYOTES (*CANIS LATRANS*) AS SENTINELS FOR BOVINE MYCOBACTERIOSIS (*MYCOBACTERIUM BOVIS*) INFECTION IN WILD CERVIDS IN AND AROUND RIDING MOUNTAIN NATIONAL PARK, MANITOBA, CANADA

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ABSTRACT: Elk (*Cervus elaphus manitobensis*) and white-tailed deer (*Odocoileus virginianus*) in the Riding Mountain National Park (RMNP) region of southwestern Manitoba have been identified as a likely wildlife reservoir of *Mycobacterium bovis,* the causative agent of bovine mycobacteriosis in livestock. The feasibility of using coyotes (*Canis latrans*) collected from trappers as a sentinel species was investigated. Retropharyngeal, mesenteric, and colonic lymph nodes and tonsils collected at necropsy from 82 coyotes were examined by bacterial culture, polymerase chain reaction (PCR), and acid-fast histopathology. *Mycobacterium bovis* was not identified in any animal by culture or PCR although *Mycobacterium avium* species were isolated. A single acid-fast organism was identified on histopathologic examination of one animal. Based on the methods used in this study, trapper-caught coyotes do not appear to be a sensitive sentinel species of *M. bovis* infection in cervids in and around RMNP.

Key words: Bovine mycobacteriosis, *Canis latrans*, cervid, coyote, *Mycobacterium bovis*, Riding Mountain National Park, sentinel.

INTRODUCTION

Elk (*Cervus elaphus manitobensis*) and white-tailed deer (*Odocoileus virginianus*) populations in and around Riding Mountain National Park (RMNP) in southwestern Manitoba, Canada (50°50′N, 100°0′W) are infected with *Mycobacterium bovis,* the causative agent of bovine mycobacteriosis. Between 1991 and 2003 there were four outbreaks of bovine mycobacteriosis in cattle involving 11 farms within this area (Lees, 2004). Based on the proximity of infected cervids to infected farms (Fig. 1) and the identification of the same *M. bovis* spoligotype in both the infected cervids and cattle, it is presumed that elk and deer in this area are a wildlife reservoir for this pathogen (Lees, 2004; Lutze-Wallace et al., 2005). The economic importance of trade restrictions resulting from this situation has prompted an effort to understand the geographic spread and host range of *M. bovis* in cervids in this area. An accurate assessment of the prevalence of infection in cervids will be needed to measure the success of management interventions. If the prevalence decreases to a very low level, it will become increasingly difficult to determine if the disease is still present. The availability of a sentinel species that could be used to detect the presence or absence of *M. bovis* in cervids would be desirable.

The term "sentinel species" refers to the use of one animal species to monitor the presence of disease in another species, the species of interest (Nugent et al., 2002). Carnivorous or omnivorous animals can serve as sentinels when there is a trophic relationship between themselves and the species of interest, through which the disease agent is passed. This strategy is

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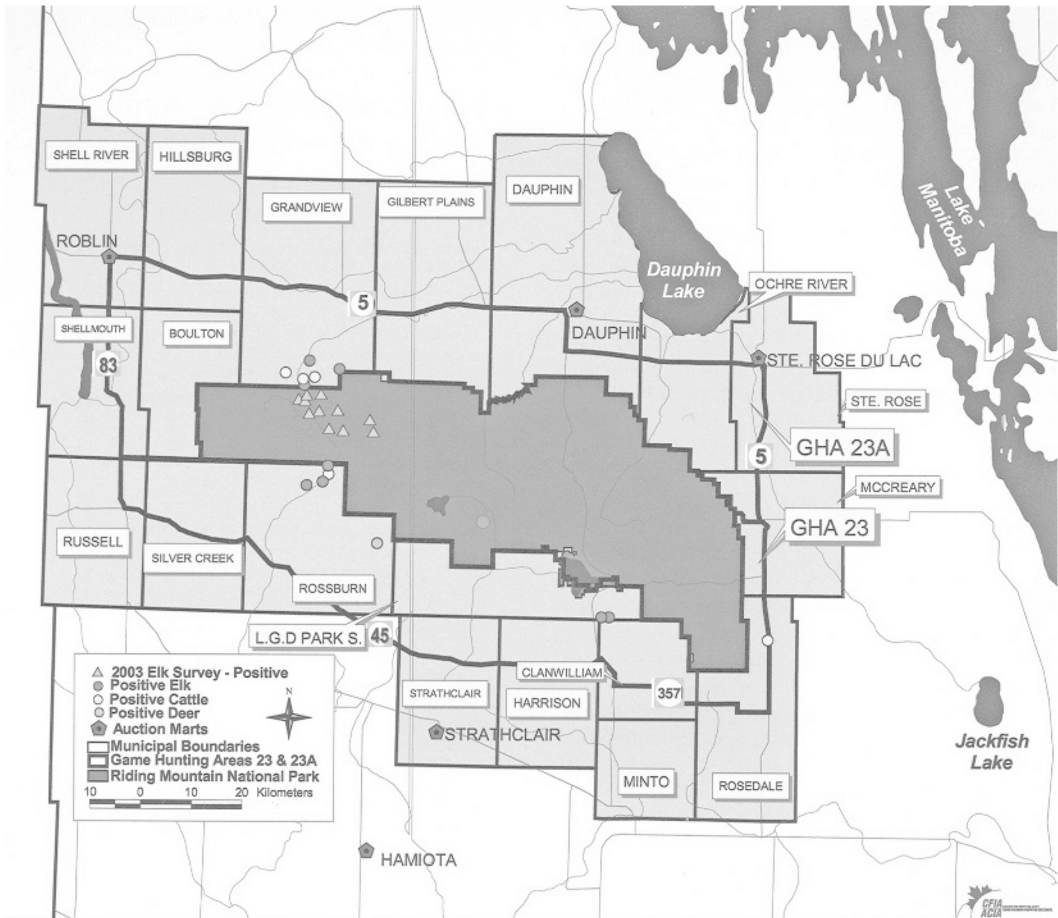

FIGURE 1. Locations of *M. bovis*–positive elk, deer, and cattle in and around Riding Mountain National Park in Manitoba, as of 2003 (from Lees, V. W. et al. 2003. Canadian Veterinary Journal 44: 830–831, with permission).

being explored in New Zealand and in Michigan, USA, as a tool to monitor for the presence of *M. bovis* in a wildlife reservoir species. In New Zealand the use of feral pigs (*Sus scrofa*) as sentinels of *M. bovis* infections in brushtail possums (*Trichosurus vulpecula*) was investigated by releasing radio-collared pigs that were subsequently recaptured and necropsied 2–9 mos later. Of the 17 pigs released, 88% were successfully recaptured, all of which were positive on culture for *M. bovis,* suggesting that pigs could be used as sentinels for the presence of bovine mycobacteriosis in possums (Nugent et al., 2002). In Michigan, where white-tailed deer are considered a wildlife reservoir for bovine mycobacteriosis, the reported prevalence of *M. bovis* in coyotes ranged from 4.8% to 30%, suggesting that they might be a good sentinel for bovine mycobacteriosis in deer (Bruning-Fann et al., 1998; Bruning-Fann et al., 2001; DeLiberto et al., 2004; O'Brien et al., 2006).

Riding Mountain National Park and the surrounding area are home to a number of large carnivores that prey and/or scavenge on cervids of the area. These include wolves (*Canis lupus*), coyotes, black bears (*Ursus americanus*), and lynx (*Lynx canadensis*). Based on an abundant population,

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perceived ease of collection, and reports from Michigan indicating significant levels of *M. bovis* infection, coyotes were thought to have potential as a sentinel species in Manitoba. The objective of this study was to investigate the feasibility of using coyotes as a sentinel species for the presence of bovine mycobacteriosis (*M. bovis*) in wild cervids, in and around RMNP.

MATERIALS AND METHODS

Coyote carcass and sample collection

Coyote carcasses were collected in the autumn and winter of 2004–05 from trappers and hunters by Natural Resource Officers of the Manitoba Department of Conservation, Regional Operations, in the municipalities of Grandview, Gilbert Plains, Rossburn, and Clan William (Fig. 1) in addition to a small number found dead by park wardens within RMNP. Skinned carcasses were stored frozen and transported to the Western College of Veterinary Medicine, where necropsies were performed. Retropharyngeal, mesenteric, and colonic lymph nodes and tonsils were collected from each animal. Portions of each lymph node were stored frozen at −70 C until processed for culture of *M. bovis* and polymerase chain reaction (PCR) analysis. The remaining portions of the lymph nodes and the tonsils were fixed in 10% neutral buffered formalin for histopathologic examination. Sex of the animal was identified, and canine teeth were collected for age determination.

Bacteriological culture

Mycobacterial culturing was conducted at the Mycobacterial Diseases Centre of Expertise, Canadian Food Inspection Agency Ottawa Laboratory Fallowfield, in Ottawa, Canada. Lymph nodes from individual coyotes were pooled and processed for inoculation. Specimen processing, decontamination, and inoculations were performed in a Class II Type A biosafety cabinet (Microzone, Ottawa, Ontario, Canada). Tissues were thoroughly examined for visible lesions; a portion of any lesion detected with surrounding tissue was placed in a disposable 50 ml conical centrifuge tube containing 10 ml 0.067 M phosphate buffer. If no visible lesions were observed, a representative piece was excised from each tissue type and placed in the buffer, for a total tissue volume of approximately 10–15 mm^3. Specimens in buffer were homogenized using a Polytron PT1200C with a 12 mm generator (Brinkmann Instruments, Westbury, New York, USA), taking care to avoid aerosols. Approximately 4 ml of the product were decanted into another 50 ml centrifuge tube to be decontaminated. Sodium hydroxide (12 mL 2% NaOH, with phenol red indicator) was dispensed into each tube, left in contact with the tissue for 12 min, and then neutralized dropwise with 2 M HCl. Sterile water was added to produce a volume of 40 ml. Tubes were centrifuged for 30 min at 3,000 × G in an Allegra 25R refrigerated centrifuge (Beckman Coulter, Inc., Fullerton, California, USA). The supernatant was decanted, leaving a button of tissue in the bottom that was used as an inoculum. All chemicals were provided by Fisher Scientific (Ottawa, Ontario, Canada).

The following selective media were inoculated: Lowenstein-Jensen (with and without sodium pyruvate), Stonebrink, 7H9 broth with antibiotics, Herrold's with mycobactin, and an antifungal cocktail (for *M. avium* subsp. *paratuberculosis*). Media were prepared in house with ingredients produced by Becton-Dickinson (Fisher Scientific) except for beef extract (Med-OX, Ottawa, Ontario, Canada) and mycobactin (Allied Monitor, Fayette, Missouri, USA). Inoculated media, except Herrold's, were incubated at 37 C and examined at 1 wk and every 2 wk thereafter, until visible colonies were observed, or for a maximum of 10 wk. Herrold's media was incubated at 39 C and observed for a maximum of 20 wk to optimize the isolation of *M. avium* subsp. *paratuberculosis.*

Smears were prepared from isolated colonies, and the Ziehl-Neelsen staining method was used to determine if acid-fast organisms were present. Any isolate that stained acid-fast was subcultured on Lowenstein-Jensen, Stonebrink and in 7H9 broth, incubated at 37 C. At 10–14 days postinoculation, the 7H9 broth was used to produce a 1:10 (concentrated) and 1:100 (dilute) inoculum to determine colony morphology, pigmentation, antibiotic susceptibility rate, and temperature of growth and to perform various biochemical assays.

If the original isolate grew on Herrold's media only, additional Herrold's (with and without mycobactin) were inoculated to determine mycobactin dependency (*M. avium* subsp. *paratuberculosis*) and incubated at 39 C.

Routine biochemical tests performed on culture isolates included arylsulphatase, heat-stable and semi-quantitative catalase, thiophen-2-carbonic acid hydrazide (TCH) susceptibility, pyrazinamidase (PZA), nitrate re-

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duction, Tween 80 hydrolysis, urease, growth on MacConkey without crystal violet, and sodium chloride tolerance. All chemicals were purchased from Fisher Scientific except TCH (Sigma Aldrich, Oakville, Ontario, Canada).

Any early indication of the presence of a possible *M. bovis* isolate (based on colony morphology, TCH sensitive, urease positive, PZA negative) was examined with the AccuProbe *M. tuberculosis* complex probe (Genprobe, San Diego, California, USA). Identification of cultures as being *M. avium* subsp. *paratuberculosis* was done by PCR using an assay targeted to an area of IS900 (Whittington et al., 1999).

Polymerase chain reaction

The PCR testing was conducted at the Western College of Veterinary Medicine. Portions of frozen lymph nodes were pooled and trimmed to a final total volume of tissue of 3–5 mm^3. DNA was extracted from these tissues with lysis buffer (100 mM NaCl, 500 mM Tris [pH 8], 10% sodium dodecyl sulfate; reagents supplied by VWR Scientific Products, Mississauga, Ontario, Canada) followed by proteinase K (0.2 mg/ml) digestion (Promega Corporation, Madison, Wisconsin, USA). The resulting product was ribolyzed in a FastPrep™ FP120 machine (ThermoSavant, Holbrook, New York, USA) at speed 6.0 for 45 sec with 100 μl of zirconium beads (Biospec Products, Inc., Bartlesville, Oklahoma, USA), followed by a second ribolyzing with an additional equal volume of phenol-chloroform (1:1). Two solvent extractions with phenol-chloroform (1:1) were performed. Nucleic acids were concentrated by precipitation in cold, salted 95% ethanol (1/10 volume 3 M sodium acetate), dried thoroughly in a vacuum, resuspended in 100 μl of sterile water, and further diluted 1:100 in sterile water.

Amplification of a 123 base-pair fragment of the IS6110 insertion sequence gene region of *Mycobacterium* sp. (tuberculosis complex) was performed using the primers IS6110-F (5′-CTCGTCCAGCGCCGCTTCGG-3′) and IS6110-R (5′- CCTGCGAGCGTAGGCGTCGG -3′). DNA was amplified in a 50 μl final reaction mixture consisting of 5 μl of 10× PCR buffer, 4 μl of 25 mM MgCl, 0.25 μl of 25 mM dNTPs, 2 μl of each primer (20 pmol), 34.5 μl of H_2O, 0.25 μl of Taq polymerase (5 U/μl), and 2 μl of sample DNA. All reagents were supplied by Fermentas Life Sciences (Ontario, Canada). The mixtures were placed in a PTC200 thermal cycler (MJ Research, Watertown, Massachusetts, USA.; licensed by Perkin-Elmer Cetus, Norwalk, Connecticut, USA). Initial incubation occurred at 94 C for 10 min followed by 72 C for 2 min, 15 sec. This was followed by 40 cycles of amplification consisting of 94 C for 45 sec and 72 C for 1 min, 25 sec. Finally, specimens were incubated at 72 C for 10 min. PCR products were separated by electrophoresis through 1.5% agarose gel and visualized with ethidium bromide using ultraviolet light. Frozen portions of pleural tissues infected with *M. bovis* from a wood bison (*Bison bison athabascae*) were used as a positive control, and PCR solution with water was used as a negative control.

Histopathology

Formalin-fixed specimens were embedded in paraffin, sectioned at 6 μm onto glass slides, and stained by the Fite's method (Sheehan and Hrapchak, 1980). All tissue sections were examined in their entirety by a single observer using a systematic scanning method at 40× power on a compound light microscope.

Maximum prevalence

Given negative results, a maximum possible prevalence of infection in a population can be estimated. This calculation was performed using WinEpiscope 2.0 software (http://www.clive.ed.ac.uk/winepiscope).

Age determination

One lower canine was removed from the mandible following submersion in boiling water for 2–3 hr. The crown of the tooth was removed with a band saw, and the remaining root was placed in 10% neutral buffered formalin for a minimum of 24 hr. Following fixation, the root was placed in a formalin-formic acid decalcification solution for 7–10 days, and then cut longitudinally in half using a scalpel. The teeth were then thin sectioned using a cryostat microtome (Microm HM500 OM, Walldorf, Germany) at 16 μm and stained with Harris's modified hematoxylin stain as described by Goodwin and Ballard (1985). Stained sections were mounted on glass slides with an aqueous mounting medium (Farrant's) and examined by a single observer using light microscopy. Coyotes were aged based on counting cementum annuli on the root of the canine tooth and adding one to the resulting number to obtain the age in years (Linhart and Knowlton, 1967). A further 6 mo was added to all coyote ages, as the animals were collected in the autumn and winter of the year. Animals with no visible cementum rings were categorized as <2.5 yr.

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RESULTS

Coyote carcass and sample collection

Eighty-two coyote carcasses were collected, transported to the Western College of Veterinary Medicine, and necropsied. Of these, 64 animals were collected from the municipalities of Grandview and Gilbert Plains, which are situated adjacent to the northern border of the park where *M. bovis* has been confirmed in wild cervids (Fig. 1) (Lees et al., 2003). Seven animals were collected in Rossburn municipality and two in Clan William municipality, which border the southern boundary of the park and in which *M. bovis* has also been confirmed in wild cervids. Two animals were collected within RMNP (these were opportunistic collections, as trapping and hunting are prohibited within park boundaries). Precise location data were not available for the remaining seven animals, except that they had been collected in one of the four municipalities. The coyotes included 46 females, 34 males, and two animals for which the sex was not determined. Grossly visible lesions consistent with *M. bovis* infection were not found in the retropharyngeal, mesenteric, or colonic lymph nodes or tonsils of any animal.

Culture

Tissue pools from individual coyotes consisting of portions of retropharyngeal, mesenteric, and colonic lymph nodes were negative on culture for *M. bovis* for all 82 animals. However, organisms of the *M. avium* complex were isolated from five animals, and *M. avium* subsp. *paratuberculosis* was isolated from three other coyotes.

Polymerase chain reaction

Tissue pools from individual coyotes consisting of portions of retropharyngeal, mesenteric, and colonic lymph nodes were negative on PCR for *M. tuberculosis* complex DNA for all 82 animals.

Histopathology

Upon examination of sections of the retropharyngeal, mesenteric, colonic lymph nodes and tonsils, one acid-fast filamentous structure, consistent with *Mycobacterium* spp., was identified in a single animal. This organism was located within the cytoplasm of a cell in the retropharyngeal lymph node.

Maximum prevalence

Using a confidence level of 95%, an estimated population size of 2,000 coyotes and a sample size of 82 coyotes, the maximum possible prevalence is 3.6%. This population size may be an overestimate, but this yields the most conservative result, with no discernible difference in the result given a larger population estimate.

Age determination

Of 81 coyotes aged, 41 were <2.5 yr old at the time of death, 23 were 2.5 yr, seven were 3.5 yr, five were 4.5 yr, three were 5.5 yr, and two were 6.5 yr at the time of death. Tooth sections were unavailable from one animal.

DISCUSSION

The collection of coyote carcasses from trappers, their transportation to the laboratory, and necropsy proved feasible in this study. Cooperation between federal and provincial agencies and the academic institution ensured successful and timely completion of the project.

Based on culture and PCR results, none of the coyotes collected in this study had retropharyngeal, mesenteric, or colonic lymph nodes that were infected with *M. bovis.* This result is strikingly different from Michigan, in which *M. bovis* has successfully been detected in coyotes by gross examination, acid-fast histopathology, and bacteriologic culture of pooled samples of parotid, submandibular, medial retropharyngeal, mediastinal, and mesenteric lymph nodes. Detection by PCR was

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not conducted on Michigan coyotes. Most gross and histologic lesions detected in Michigan occurred in the mesenteric lymph nodes (Bruning-Fann et al., 2001); thus it is expected that *M. bovis* would have been detected had it been present in the coyotes from around RMNP. Eight coyotes were culture positive for other *Mycobacterium* spp., and positive controls of the PCR technique were conducted, so the negative results reported in this study are presumed to be valid.

There are a number of possible explanations for our failure to identify infected coyotes. One is that the prevalence was too low to be detected with the number of coyotes examined. If the prevalence of infection was lower than 3.6%, the maximum prevalence equation would predict that a sample size of 82 coyotes was insufficient to have detected *M. bovis* infection. Further, the sampled coyotes included 78% aged as ≤2.5 years. Older animals would have had a greater opportunity to be exposed to cervids or carcasses and therefore would have had more opportunity to become infected. In addition, infections caused by *Mycobacterium* spp. are typically chronic and progressive, so that older animals may have larger lesions that are more likely to be detected (Clifton-Hadley et al., 2001). Given the age structure found in this study, a much larger sample size may be advisable when using coyote carcasses salvaged from trappers as the collection technique.

Alternative reasons to explain the lack of positive results in this study need to be considered. It is possible that some coyotes had no access to areas populated by *M. bovis*–infected cervids. Although the size of home ranges of coyotes in this area is unknown, this explanation seems less likely given that all the coyotes came from municipalities in which infected cervids or cattle have occurred or are directly adjacent to areas of the park in which infected cervids had been found. Ideally, coyotes would have been collected within the park, but, because of the use of primarily salvaged, trapper-caught carcasses, this was precluded.

Although white-tailed deer have tested positive for *M. bovis* in this area, the primary reservoir species appears to be elk, an animal that is too large as an adult to be preyed upon by coyotes. Riding Mountain National Park is home to wolves and bears that may compete with coyotes for cervid prey or carcasses. If coyotes are able to scavenge on cervids killed by wolves, they are unlikely to obtain portions of the visceral organs, such as the lungs and lymph nodes, which are among the prime sites of mycobacteriosis in cervids (Clifton-Hadley et al., 2001). In Michigan, where the prevalence of *M. bovis* infection in coyotes has been reported to range from 4.8% to 30% (Bruning-Fann et al., 1998; Bruning-Fann et al., 2001; DeLiberto et al., 2004; O'Brien et al., 2006), coyotes may have more opportunity for exposure for two related reasons. Although the prevalence of *M. bovis* infection in cervids in northeastern lower Michigan is similar to that in the municipalities of Grandview and Rossburn (Lees et al., 2003; O'Brien et al., 2006), the density of deer in these two areas is dramatically different. With approximately 10 times as many deer per square kilometer in Michigan as in Manitoba, based on published deer population estimates and state/province areas (www.michigan.gov/dnr; www.gov.mb.ca/conservation), the opportunity for a coyote to contact an infected deer is much greater in Michigan. The hunting pressure in Michigan is also much greater, providing more potential for coyotes to scavenge on viscera discarded by hunters that could contain infected material.

Trapper-caught coyotes do not appear to be a good sentinel species for bovine mycobacteriosis in cervids in the area of RMNP when utilizing the methods employed in this study. Other carnivores (wolves, black bears, and lynx) might be considered. Based on natural history, wolves, which have a more direct trophic

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relationship with elk, could be an appropriate choice. Two wolves found dead within the park in 1978 had greatly enlarged mesenteric lymph nodes that were culture positive for *M. bovis* (Carbyn, 1982). However, the number of wolves present in the area is much lower than the number of coyotes, and most wolves are within RMNP and are not taken regularly by trappers or hunters. Bears may be the next logical species to warrant investigation since they prey on elk calves and scavenge on carcasses, are transboundary animals, and are hunted in the area, thus providing access to sufficient numbers of carcasses.

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LITERATURE CITED

BRUNING-FANN, C. S., S. M. SCHMITT, S. D. FITZGERALD, J. S. FIERKE, P. D. FRIEDRICH, J. B. KANEENE, K. A. CLARKE, K. L. BUTLER, J. B. PAYEUR, D. L. WHIPPLE, T. M. COOLEY, J. M. MILLER, AND D. P. MUZO. 2001. Bovine tuberculosis in free-ranging carnivores from Michigan. Journal of Wildlife Diseases 37: 58–64.

———, ———, ———, J. B. PAYEUR, D. L. WHIPPLE, T. M. COOLEY, T. CARLSON, AND P. FRIEDRICH. 1998. *Mycobacterium bovis* in coyotes from Michigan. Journal of Wildlife Diseases 34: 632–636.

CARBYN, L. N. 1982. Incidence of disease and its potential role in the population dynamics of wolves in Riding Mountain National Park, Manitoba. *In* Wolves of the world: Perspectives of behavior, ecology, and conservation, F. H. Harrington and P. C. Paquet (eds.). Noyes Publications, Park Ridge, New Jersey, pp. 106–116.

CLIFTON-HADLEY, R. S., C. M. SAUTER-LOUIS, AND I. W. LUGTON. 2001. Mycobacterial diseases: *Mycobacterium bovis* infections. *In* Infectious diseases of wild mammals, E. S. Williams and I. K. Barker (eds.). Iowa State University Press, Ames, Iowa, pp. 340–371.

DELIBERTO, T. J., K. C. VERCAUTERAN, AND G. W. WITMER. 2004. The ecology of *Mycobacterium bovis* in Michigan. *In* Michigan Bovine Tuberculosis Eradication Project—2004 Activities Report and Conference Proceedings, 9–10 June, East Lansing, Michigan, pp. 1–36.

GOODWIN, E. A., AND W. B. BALLARD. 1985. Use of tooth cementum for age determination of gray wolves. Journal of Wildlife Management 49: 313–316.

LEES, V. W. 2004. Learning from outbreaks of bovine tuberculosis near Riding Mountain National Park: Applications to a foreign animal disease outbreak. Canadian Veterinary Journal 45: 28–34.

———, S. COPELAND, AND P. ROUSSEAU. 2003. Bovine tuberculosis in elk (*Cervus elaphus manitobensis*) near Riding Mountain National Park, Manitoba, from 1992 to 2002. Canadian Veterinary Journal 44: 830–831.

LINHART, S. B., AND F. F. KNOWLTON. 1967. Determining age of coyotes by tooth cementum layers. Journal of Wildlife Management 31: 362–365.

LUTZE-WALLACE, C., C. TURCOTTE, M. SABOURIN, G. BERLIE-SURUJBALLI, Y. BARBEAU, D. WATCHORN, AND J. BELL. 2005. Spoligotyping of *Mycobacterium bovis* isolates found in Manitoba. Canadian Journal of Veterinary Research 69: 143–145.

NUGENT, G., J. WHITFORD, AND N. YOUNG. 2002. Use of released pigs as sentinels for *Mycobacterium bovis*. Journal of Wildlife Diseases 38: 665–677.

O'BRIEN, D. J., S. M. SCHMITT, S. D. FITZGERALD, D. E. BERRY, AND G. J. HICKLING. 2006. Managing the wildlife reservoir of *Mycobacterium bovis:* The Michigan, USA, experience. Veterinary Microbiology 112: 313–323.

SHEEHAN, D. C., AND B. B. HRAPCHAK. 1980. Theory and practice of histotechnology. 2nd Edition. C. V. Mosby Company, St. Louis, Missouri, 481 pp.

WHITTINGTON, R. J., L. REDDACLIFF, I. MARSH, AND V. SAUNDERS. 1999. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in formalin-fixed paraffin-embedded intestinal tissue by IS900 polymerase chain reaction. Australian Veterinary Journal 77: 392–397.

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