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Epizootiologic Survey of Mycobacterium Bovis in Wildlife and Farm Environments in Northern Michigan

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EPIZOOTIOLOGIC SURVEY OF *MYCOBACTERIUM BOVIS* IN WILDLIFE AND FARM ENVIRONMENTS IN NORTHERN MICHIGAN

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ABSTRACT: Bovine tuberculosis (bovine TB), caused by *Mycobacterium bovis*, has reemerged in northern Michigan, USA, with detections in white-tailed deer (*Odocoileus virginianus*) in 1994 and in cattle in 1998. Since then, significant efforts have been directed toward reducing deer densities in the area in the hopes of reducing the bovine TB prevalence rate in deer and eliminating spillover of the disease into cattle. Despite the success of the efforts to reduce deer densities, additional cattle herds have become infected. Other mammals can be infected with *M. bovis*, and some carnivores and omnivores had been found to be infected with the disease in northern Michigan, USA. We conducted a multiyear surveillance effort to detect bovine TB in wild species of mammals in the Michigan, USA, outbreak area. From 2002 to 2004, tissue samples from 1,031 individual animals of 32 species were collected, processed, and cultured for *M. bovis*. Only 10 (1.0%) were culture-positive for *M. bovis* (five raccoons [*Procyon lotor*], four opossums [*Didelphis virginiana*], and one grey fox [*Urocyon cinereoargenteus*]). We also found two raccoons and four opossums to be positive for *Mycobacterium avium*. We collected 503 environmental samples from cattle farms recently identified as bovine TB positive; none yielded positive *M. bovis* culture results. Finally, we used infrared cameras to document wildlife use of four barns in the area. Many avian and mammalian species of wildlife were observed, with raccoons being the most commonly observed species. This surveillance study identified no new wildlife species that should be considered significant reservoirs of bovine TB in the outbreak area in northern Michigan, USA. However, the relatively high, apparent bovine TB prevalence rates in some carnivorous and omnivorous species, their relatively long life spans, and their frequent use of barns, suggests that removal of raccoons, opossums, foxes, and coyotes (*Canis latrans*) should be considered when a newly infected farm is depopulated of cattle.

Key words: Bovine tuberculosis, disease transmission, environmental contamination, farm management, *Mycobacterium bovis*, prevalence rate, surveillance, wildlife management.

INTRODUCTION

Bovine tuberculosis (bovine TB) is a bacterial disease caused by *Mycobacterium bovis*, which occurs nearly worldwide (O'Reilly and Daborn, 1995; Clifton-Hadley et al., 2001). Many mammalian species, including humans, are susceptible to bovine TB (O'Reilly and Daborn, 1995; Whipple and Palmer, 2000; Clifton-Hadley et al., 2001; Coleman and Cooke, 2001; de Lisle et al., 2001). Bovine TB infection in humans in the United States was relatively common until the pasteurization of milk (Whipple and Palmer, 2000). In 1979, Michigan, USA, was declared free of bovine TB (Whipple and Palmer, 2000). Bovine TB has reemerged in northern Michigan, USA, when it was first detected in white-tailed deer (*Odocoileus virginianus*) in 1994 and in domestic cattle herds in 1998 (Schmitt et al., 2002). Although cases of bovine TB have been found in other domestic and wild species in Michigan, USA, only white-tailed deer have been identified as a potential disease reservoir (Schmitt et al., 2002; O'Brien et al., 2006). Other infected wildlife species

are considered to be spillover or dead-end hosts (Whipple and Palmer, 2000; Payeur et al., 2002; O'Brien et al., 2006). In other countries, however, other wild species are the primary reservoirs for this disease (e.g., the European badger [*Meles meles*] in the United Kingdom and the Australian brush-tail possum [*Trichosurus vulpecula*] and the European ferret [*Mustela furo*] in New Zealand; O'Reilly and Daborn, 1995; Clifton-Hadley et al., 2001; Corner, 2006). A survey of various wild mammals and birds in western Texas, USA, failed to identify any new reservoirs of bovine TB, although most of the animals captured and tested were either Rock Pigeons (*Columba livia*) or house mice (*Mus musculus*; Pillai et al., 2000). Knowledge of the number and types of bovine TB reservoirs and routes of transmission in an outbreak area is essential for the identification and implementation of appropriate and effective management practices (Kaneene et al., 2002).

The main objective of this study was to conduct wildlife surveillance for bovine TB in the Michigan, USA, outbreak area to detect other potential wildlife reservoirs of the disease. A second objective of the study was to assess potential environmental contamination of farms with *M. bovis*. These bacteria can persist for months under certain environmental conditions; in particular, moist, cool conditions are conducive to survival (Duffield and Young, 1985; Jackson et al., 1995; Fine, 2006). Knowledge of the use of farms and barns by wildlife has implications for the development of appropriate management actions (Garnett el al., 2003; Kaneene et al., 2002; Hill, 2005). Hence, a third objective of the study was to determine the frequency of wildlife use of barns in the outbreak area. This component of the surveillance study was conducted because many of the barns in the bovine TB core area are visited or inhabited by raccoons, opossums, and other wildlife, and species of carnivores and omnivores can have relatively high (≥3%) bovine TB prevalence rates (Bruning-Fann et al., 2001; Schmitt et al., 2002; O'Brien et al., 2006). Cameras in barns in the United Kingdom revealed considerable use of livestock feed and water by European badgers, some of which, later tested positive for *M. bovis* (Garnett et al., 2003).

MATERIALS AND METHODS

Study area

This study was conducted in northeastern lower Michigan, USA. The 1,550-km^2 area includes portions of Montmorency, Alpena, Oscoda, and Alcona counties. A corner of each of these four counties meets at 44°51′N, 83°53′W. This area is considered to be the core area of the bovine TB outbreak area in Michigan, USA. Lands in the area are largely under private ownership and consist of farms (including some having had previously infected cattle herds and some never having infected herds), private deer hunting clubs, and some parcels of land managed by the Michigan Department of Natural Resources (DNR). Bovine TB–positive, hunter-harvested deer have been found widely distributed over the area's private and public hunting lands (Patrick 2003). Aside from areas cleared for agricultural crops or pasture, habitat types included upland hardwood stands (*Quercus alba*, *Acer rubrum*, and *Acer saccharum*), aspen stands (*Populus tremuloides* and *Populus grandidentata*), hardwood/aspen mixed stands, upland conifer stands (*Pinus glauca*, *Pinus banksiana*, and *Pinus resinosa*), hardwood/conifer mixed stands, and lowland conifer forests/swamps (*P. glauca*, *Pinus mariana*, *Thuja occidentalis*, *Abies balsamea*, and *Latrix laricinea*; Dickman, 2004). The area is relatively level with elevations in the range of 150–390 m above sea level (Williams, 1992). The mean annual temperature is 6.6 C, the mean rainfall is 72.5 cm, and there is a mean snowfall of 175 cm (Hughey, 2003).

Field methods

Twelve wildlife species were targeted for the initial sampling, with a goal of testing at least 60 individuals of each species: meadow vole (*Microtis pennsylvanicus*), deer/white-footed mouse (*Peromyscus* spp.), house mouse (*Mus musculus*), porcupine (*Erethizon dorsatum*), Virginia opossum (*Didelphis virginiana*), eastern cottontail (*Sylvilagus floridanus*), snowshoe hare (*Lepus americanus*), eastern chipmunk (*Tamias striatus*), red squirrel

TABLE 1. Bovine tuberculosis (TB) culture results from wildlife collected in the bovine TB core area of northern Michigan, USA, 2002–2004.

Common name/scientific name	Samples	Negative	Acid fast (+)	*M. bovis* (+)	Acid-fast organism
North American badger/*Taxidea taxus*	4	4	0	0	
North American beaver/*Castor canadensis*	61	61	0	0	
Bobcat/*Felis rufus* and/*Lynx rufus*	3	3	0	0	
Coyote/*Canis latrans*	2	2	0	0	
Deer mouse/*Peromyscus maniculatus*	24	24	0	0	
Domestic cat/*Felis catus*	10	10	0	0	
Domestic rabbit/*Oryctolagus cuniculus*	1	0	1	0	*Nocardia* spp.
Eastern chipmunk/*Tamias striatus*	66	66	0	0	
Eastern cottontail/*Sylvilagus floridanus*	41	40	1	0	Not identified
Eastern gray squirrel/*Sciurus carolinensis*	26	26	0	0	
Eastern mole/*Scalopus aquaticus*	1	1	0	0	
Fox squirrel/*Sciurus niger*	17	17	0	0	
Gray fox/*Urocyon cinereoargenteus*	1	0	1	1	
House mouse/*Mus musculus*	62	62	0	0	
Meadow jumping mouse/*Zapus hudsonicus*	7	7	0	0	
Meadow vole/*Microtus pennsylvanicus*	77	77	0	0	
Muskrat/*Ondatra zibethicus*	5	5	0	0	
Northern flying squirrel/*Glaucomys sabrinus*	1	1	0	0	
Porcupine/*Erethizon dorsatum*	71	69	2	0	Not identified
Raccoon/*Procyon lotor*	203	191	8	5	*M. avium* (n=2), *M. fortuitum*
Red squirrel/*Tamias striatus*	58	58	0	0	
Red-backed vole/*Clethrionomys gapperi*	3	3	0	0	
Snowshoe hare/*Lepus americanus*	23	23	0	0	
Southern bog lemming/*Synaptomys cooperi*	1	1	0	0	
Southern flying squirrel/*Glaucomys volans*	3	3	0	0	
Striped skunk/*Mephitis mephitis*	46	44	1	0	*M. fortuitum*
13-lined ground squirrel/*Spermophilus tridecemlineatus*	4	4	0	0	
Virginia opossum/*Didelphis virginianus*	135	125	9	4	*M. avium* (n=4), *Nocardia* spp.
White-footed mouse/*Peromyscus leucopus*	66	65	1	0	*Nocardia* spp.
White-tailed deer/*Odocoileus virginianus*	2	2	0	0	
Woodchuck/*Marmota monax*	10	10	0	0	
Woodland vole/*Microtus pinetorum*	3	3	0	0	
Total	1,037	1,007	24	10	

(*Tamiasciurus hudsonicus*), North American beaver (*Castor canadensis*), raccoon (*Procyon lotor*), and striped skunk (*Mephitis mephitis*). Other species were collected opportunistically, as authorized under the collecting permit. A complete list of the species collected can be found in Table 1. The species targeted for collection represent a diverse taxonomic group of medium- to small-sized mammals that are relatively common in the area and around farms (Hill, 2005; Clarke et al., 2007) and whose densities were sufficient to allow collection of adequate numbers. Animals were collected with the use of traps (cage traps, snap traps, leg-hold traps, and snares) and by shooting, all under a scientific collecting permit issued by the DNR Wildlife Division. Trapped animals were euthanized by a gunshot to the brain. Fresh vehicle-killed mammals were also collected. The collection of animals began in May 2002 and was completed in August 2004. Animals were necropsied in an aseptic mobile laboratory set up for that purpose at the Michigan Department of Agriculture (MDA) facility in Atlanta, Michigan, USA, near the outbreak area. We removed lymph node tissues (tonsillar/soft palate area, retropharyngeal, tracheobronchial, mediastinal, mesenteric), a small (generally $\geq 5 \times 5$ mm) piece of lung tissue, and any lesions observed. Sample selection was based on the premise that bovine TB can be

manifested in various tissues/organs, depending in part on route(s) of exposure (Whipple et al., 1996; Palmer et al., 2003; Clarke et al., 2007). Pooling lymph node samples has been shown to result in a higher detection rate of TB infection in ferrets (de Lisle et al., 2004). All tissues from an individual animal were pooled and placed into a labeled, Whirl-Pak® specimen plastic bag (Nasco International, Fort Atkinson, Wisconsin, USA), which was then placed in a second zipper-sealed plastic bag. All samples were kept refrigerated or on ice until delivered to the biosafety level III laboratory at the Michigan State University Diagnostic Center for Population and Animal Health (MSU DCPAH) for mycobacterial isolation and identification. Samples that could not be delivered within a few days were frozen at −20 C until delivery.

Environmental samples were collected from three newly diagnosed TB positive farms between December 2003 and September 2004. Up to 20 samples each of soil, water, livestock bedding, livestock feed, hay, pasture grass, livestock feces, deer feces, and carnivore feces were collected from each farm. The samples (20 g each) were placed in labeled, sterile bottles or zipper-sealed plastic bags and refrigerated or stored on ice until delivery to the MSU DCPAH facility for bacterial isolation and identification.

The use of barns by wildlife was investigated using infrared video camera systems (Infrared B/W CCD, SuperCircuits, Austin, Texas, USA) that recorded animal activity in barns 24 hr/day from four different vantage points. The vantage points included views across large barn doors that were typically left open, views along cattle feeding troughs, views in hay lofts, views along cattle holding pens, and views in grain storage areas and/or hay bales. We collected seasonal video footage from four barns on northeastern Michigan farms from June 2003 through June 2004. In any given month, cameras were operated 24 hr/day for at least 3 consecutive days. For purposes of data compilation and summarization, we used tabulated observations from videotapes for June, August, and September 2003 and February 2004. Data on all animals that could be identified on tape were recorded by date, farm, species, and activity/behavior.

Laboratory methods

Wildlife tissue samples received at the laboratory were stored at −20 C (or at −70 C for extended periods) until processing. Individual samples were thawed, processed, and decontaminated following standard protocols (Kent and Kubica, 1985) and following protocols adapted from those established for preparing white-tailed deer tissue samples for mycobacterial culture (de Lisle et al., 2002). Briefly, samples were placed in individual, sterilized quart jars, covered with phenol red broth and then mixed by securing a blade unit and gasket on the jars, inverting and blending for 30 sec or until liquefied. Samples were then transferred to 50-ml centrifuge tubes and decontaminated with a sodium-hypochlorite-sodium hydroxide method. Samples were then spun in a refrigerated centrifuge at 1,000 × G for 20 min. Enriched Middlebrook broth, 7H12B, was then inoculated with 200 µl of sample suspension in the BACTEC 460 system (Becton-Dickinson Diagnostic Systems, Franklin Lakes, New Jersey, USA). Samples were also inoculated onto solid media slants and plates containing modified Middlebrook 7H11 agar (Becton-Dickinson) with sodium pyruvate (DCPAH, Lansing, Michigan, USA), Lowenstein Jensen (DCPAH) and 7H11 selective plates (Becton-Dickinson). The solid media tubes and plates were incubated at 37 C and examined weekly until colonies were observed or until an incubation period of 12 wk was complete, at which time tubes and plates with no growth were discarded. Colonies from solid media and liquid culture bottles that showed positive signals were examined with a Ziehl-Neelsen acid-fast staining technique. Acid-fast–positive bacteria were screened using AccuProbe *Mycobacterium tuberculosis* complex nucleic acid probes (Gen-Probe, San Diego, California, USA) to determine whether the acid-fast bacteria were of the *M. tuberculosis* complex. *Mycobacterium bovis* was distinguished from *M. tuberculosis* isolates using biochemical tests and high-performance liquid chromatography (Butler et al. 1991; Kent and Kubica, 1985).

The environmental samples (livestock feed, feces, water, etc.) were processed with protocols developed by optimizing the procedures for processing corn, hay, soil, and water samples for mycobacterial culture (Fine, 2006) and adapted for the other types of environmental samples collected, including the cattle and carnivore feces. The optimized procedures for processing environmental samples used the CB-18™ TB Culture Kit with Lytic Decon™ II (Integrated Research Technology, Quest Diagnostics, Baltimore, Maryland, USA). Briefly, solutions and reagents necessary for the CB-18 processing were made according to the procedures outlined in the instruction booklet. Approximately 5 g of the solid substrates and 7.5 ml of liquid substrates were transferred to 3×7-inch Whirl-Pak bags

(soil and feces), sterilized pint jars (feed and vegetation), or 50-ml conical centrifuge tubes (water and liquid samples) for further processing. Sterile water (7.5 ml) and 5 ml of liquefaction solution (trisodium citrate dehydrate and *N*-acetyl-L-cysteine [NALC]) were added to the solid substrates. Samples were then pulverized and homogenized and allowed to settle for 30 min.

The top 5 ml of fluid from each sample was removed and transferred to a 50-ml conical tube and prepared for mycobacterial culture by first adding 10 ml of decontamination solution (20× Tris-citrate buffer, CB-18 stock, or NALC and water) and incubating at 37 C for 75 min. Samples were then diluted with sterile water and centrifuged at 3,000×G for 20 min to harvest the pellet. One milliliter of 2× resuspension solution (10× enzyme stock, *Trichoderma harzianum* extract, lysozyme and *Lysobacter* extract, and NALC) was added to each sample and they were incubated for 45 min at 37 C. CB-18–processed samples were inoculated onto solid media slants and plates containing modified Middlebrook 7H11 agar with sodium pyruvate and 7H11 selective plates. Solid media slants and plates were incubated at 37 C for 8–12 wk and examined weekly for colony formation. Positive cultures and colonies on solid media were subjected to an acid-fast smear analysis to confirm the presence of acid-fast bacteria, using standard protocols for slide preparation, staining, and examination (Kent and Kubica, 1985). If acid-fast bacteria were found, they were processed as described above for tissue samples.

RESULTS

By October 2006, results of 1,031 cultured samples were received (99% of submissions) from the MSU diagnostic laboratory (Table 1). Six samples were lost or contaminated and could not be processed. Four opossums tested positive for *M. bovis*, four were positive for *M. avium*, and one was positive for *Nocardia* spp. (Table 1). The bovine TB-positive opossums (all females) were collected from two farms and a deer hunt club. These animals were collected in June ($n=2$) and September ($n=2$) of 2002. The farms, about 1.2 km apart, had been previously diagnosed as having infected cattle and had those cattle depopulated. The opossum from the deer hunt club was about 5.2 km from those farms. Five raccoons tested positive for *M. bovis*, two were positive for *M. avium*, and one was positive for *M. fortuitum* (Table 1). The bovine TB-positive raccoons (two females and three males) were collected from two deer hunt clubs and DNR state lands. The raccoons had all been collected in June 2002. These animals were collected a considerable distance from the farms that had infected opossums, about 11.2–25.6 km distant. A male gray fox (*Urocyon cinereoargenteus*), collected from a farm, was positive for *M. bovis*. We believe this may be the first documentation of *M. bovis* in a gray fox in Michigan. That farm had been previously diagnosed as having infected cattle and had those cattle depopulated. The farm was outside and very distant (about 280 km northwest) from the center of the outbreak area, where the other nine positive animals were collected. The smallest rectangle that encompassed all nine positive animals from the core area was 27.4 by 25.8 km on a side (707 km^2). The distribution of the four TB-positive opossums was rather tightly clumped (10.4 km^2), whereas the distribution of the five raccoons was much more widely dispersed (416 km^2). Other acid-fast-bacteria positive animals included one skunk (*M. fortuitum*), one domestic rabbit (*Nocardia* spp.), one eastern cottontail (acid-fast bacteria unidentified, but not *M. bovis*), one white-footed mouse (*Nocardia* spp.), and two porcupines (acid-fast bacteria unidentified, but not *M. bovis*).

The overall prevalence of bovine TB among animals from this surveillance study was 1.0% (10 TB-positive animals out of 1,031 tested specimens). Raccoons showed a prevalence of 2.5% (five of 199; 95% confidence limit, 0.3–4.7%). Prevalence in opossums was 3.0% (four of 134; 95% confidence limit, 0.1–5.9%).

Culture results were received for 455 environmental samples (90.5% of submissions; Table 2). All were negative for *M. bovis*. One water sample was positive for *M. fortuitum*. Remaining submitted samples were not cultured for *M. bovis*

TABLE 2. Number and type of environmental samples cultured for bovine tuberculosis (TB) from the bovine TB core area of northern Michigan, USA, 2003–2004. No samples were positive for bovine TB.

Sample type	Farm 111	Farm 112	Farm 113	All farms
Sampling dates	4–10 December 2003	2–5 April 2004	4–9 July 2004	
Cattle feed (hay)	20	8	18	46
Cattle feed (corn)	0	0	8	8
Cattle straw bedding	20	15	20	55
Cattle feces	20	13	20	53
Water	20[a]	35	35	90
Soil	20	15	41	76
Carnivore feces	20	16	20	56
Deer feces	20	10	2	32
Pasture forage	20	0	17	37
Deer urine	2	0	0	2
Total	162	112	181	455

[a] One water sample was positive for *M. fortuitum*.

because of the length of time the samples had been frozen.

At least 17 species of animals were observed in the four barns surveyed (Table 3). Because we could not always identify the species of small bird or rodent, the total number of animal species using the barns may have been greater. Wild species observed in the barns included raccoons, skunks, opossums, eastern cottontails, tree squirrels, small rodents (mice, rats, and chipmunks), small birds (including Swallows [*Hirundo* spp.], sparrows [*Spizella* spp.], European Starlings [*Sturnus vulgaris*], Blue Jays [*Cyanocitta cristata*], Mourning Doves [*Zenaida macroura*]), and Wild Turkeys (*Meleagris gallopavo*). Also observed were cats, dogs, chickens, horses, and cattle. In 17.4% of the cases, animals were observed while cattle were present (Table 3). Raccoons were the single most-commonly observed species of wildlife in barns (Table 3). The number of animal observations remained high (500–600) in June, August, and September but dropped to fewer than 200 in February, presumably because many animals had migrated south or were in hibernation. Many behaviors were observed. For example, for raccoons, behaviors observed (in descending order of occurrence) included walk/run, stand/sit, feed, sniff, interact with same species, climb/jump, lay/sleep, interact with cattle, groom, and defecate. We recorded a raccoon face-to-face with a cow at a

TABLE 3. Total wildlife and dog/cat observations in barns using infrared video cameras for a 72-hr period by month in northern Michigan, USA, 2003–2004. Numbers in parentheses are numbers of animals observed when cattle were present.

Species	June 2003	August 2003	September 2003	February 2004	Totals
Raccoons	315 (1)	324 (180)	69 (15)	0	708 (195)
Birds	184 (79)	112 (0)	50 (14)	0	346 (93)
Rodents	10 (0)	82 (8)	477 (8)	11 (0)	580 (16)
Cats	84 (3)	0	7 (1)	148 (2)	239 (6)
Opossums	1 (0)	1 (0)	8 (0)	23 (21)	33 (21)
Skunks	9 (1)	2 (1)	21 (9)	0	32 (11)
Dogs	13 (1)	0	1 (0)	4 (0)	18 (1)
Squirrels	0	5 (0)	3 (0)	0	8 (0)
Unknown	2 (0)	0	1 (0)	0	3 (0)
Totals:	618 (85)	526 (189)	637 (47)	186 (23)	1,967 (343)

distance of about 0.3 m; this raccoon then tasted a salt lick that the cow had been licking about 20 min earlier. For opossums, behaviors observed (in descending order of occurrence) included walk/run, sniff, stand/sit, and climb/jump. For skunks, behaviors observed (in descending order of occurrence) included walk/run, sniff, stand/sit, groom, and interact with other species.

DISCUSSION

This study represents the largest effort to conduct targeted surveillance of wildlife species, other than white-tailed deer, in the northern Michigan, USA, bovine TB outbreak area. Although this surveillance study identified no new wildlife species that should be considered significant reservoirs of bovine TB, it provides information about the prevalence of bovine TB in a number of wild carnivorous and omnivorous species. These "spillover" species have a relatively long life span, sizable home ranges, and frequently use barns on cattle farms in the region and should, therefore, be considered for removal as part of the farm disinfection protocol for bovine TB-positive farms.

Prevalence of bovine TB for raccoons in this study (2.5%) was similar to that found by the Michigan DNR (2.4%, eight of 333) as of July 2003, which sampled carnivores and omnivores opportunistically throughout a larger area (Schmitt et al., 2002; O'Brien et al., 2006). Prevalence of bovine TB for opossums in this study (3.0%) was higher than the prevalence of bovine TB for opossums found by the Michigan DNR (0.5%, two of 379). Because all samples from our study were collected within the core area, higher prevalence rates were expected. O'Brien et al. (2006) also reported a 10% prevalence of bovine TB in red foxes (*Vulpes vulpes*), but did not report any bovine TB-positive gray foxes.

Interestingly, our last TB-positive animal was collected relatively early in the study (September 2002). The overall prevalence of bovine TB has dropped from about 4% to about 2% in white-tailed deer in the core area since 1995 (O'Brien et al., 2002; Schmitt et al., 2002; O'Brien et al., 2006). It would be expected that prevalence is dropping in other wildlife species as well because they are considered spillover species, that is, they are exposed and infected primarily by scavenging on dead, infected deer carcasses (Bruning-Fann et al., 2001; O'Brien et al., 2006).

Fine (2006) collected and processed environmental samples (soil, feed, grass, manure, and water) from 10 farms with bovine TB-positive cattle and five sites outside farms where wildlife with confirmed bovine TB infection had been captured. Acid-fast bacteria were cultured from several of these samples, but bovine *M. bovis* was not detected. That result was consistent with our findings. The failure to identify *M. bovis* from environmental samples from these sites indicates that the *M. bovis* may be rare or patchily distributed in the environment, thus making it very difficult to detect even on newly infected farms. A procedure for concentrating the bacteria from larger environmental samples is likely needed before routine environmental sampling can be effectively used as a complement to surveillance of animals for bovine TB presence.

We documented a large number of wild mammal species and domestic animals visiting barns and exhibiting a wide array of behaviors, including close contact with cattle, drinking, using cattle feed troughs, and licking salt blocks. Such situations of close contact and shared feed and water present the opportunity for disease transfer (Palmer et al., 2001; Kaneene et al., 2002; Garnett et al., 2003; Palmer et al., 2004; Hill 2005). Hill (2005) documented a sizable number of wildlife occurrences outside barns in Michigan, USA, some of which included close contact with cattle and suggested that this situation may be a potential source of TB transmission be-

tween animals. Because of the home-range sizes and dispersal capabilities of medium- and large-sized mammals, one infected animal could easily visit several farms.

A large number of birds visited and used the barns (Table 3). Although bovine TB surveillance conducted in west Texas, USA, did not identify any infected birds (Pillai et al., 2000), there has been discussion at TB scientific meetings on bovine TB in Michigan, USA (Patrick, 2003) about the possible physical transfer of disease organisms on the feet of birds. Although we did not collect and test birds for bovine TB infection, Fitzgerald et al. (2003a) reported that Rock Pigeons can become infected under experimental conditions and can shed the bacteria in feces. Hill (2005) reported a sizable number of visits to Michigan, USA, farms by Wild Turkeys, including close contact with cattle.

In summary, this study did not identify any new wildlife species as significant disease reservoirs or vectors of bovine TB in the outbreak area in northern Michigan, USA. In this regard, our study confirms the findings of the Michigan DNR: Aside from deer and cattle, the occurrence of bovine TB in mammals in the outbreak area seems to be limited to carnivores and omnivores (black bear [*Ursus americanus*], bobcat [*Felis rufus/ Lynx rufus*], domestic cat, coyote (*Canis latrans*), raccoon, fox, and opossum; Bruning-Fann et al., 2001; Schmitt et al., 2002; O'Brien et al. 2006; VerCauteren et al., 2008). Presumably, these animals become infected by consuming infected prey or by scavenging on infected carcasses. Once infected, these individuals may infect conspecifics based on particular behavioral patterns related to shared food and water sources, close contact (mutual grooming and breeding), and shared areas (such as when several raccoons use one winter den; Merritt, 1987). European badgers have also been reported to infect each other with *M. bovis* by biting (Jenkins et al., 2008).

Concern has been expressed at scientific meetings on bovine TB in Michigan, USA (Patrick 2003), about the possible role of raccoons and opossums in the epidemiology of bovine TB in the outbreak area (Patrick, 2003). Our results confirm these concerns based on bovine TB prevalence ($\geq$2.5%) in these species and their frequent occurrence in barns. Opossums and raccoons were experimentally infected with *M. bovis*, and some individuals shed the bacteria (Fitzgerald et al., 2003b; Palmer et al., 2002). However, we do not know whether naturally infected animals of these species would shed the bacteria in the wild or merely serve as dead-end or spillover hosts. VerCauteren et al. (2008) recently reported prevalence rates in coyotes in the outbreak area as high as 52%. Because of the relatively high TB prevalence rates in some carnivorous and omnivorous species, their relatively long life span, their frequent use of barns, and the possibility that some of them may be shedding the bacteria, when a newly infected farm is depopulated of cattle and disinfected, removal of raccoons, opossums, foxes, and coyotes should be considered. Wilkins et al. (2008) did not detect bovine TB in dogs and cats residing at infected cattle farms in Michigan, USA, but recommended that pets also be depopulated if they had been heavily exposed to infected cattle. Although we did not detect *M. bovis* in the rodents we sampled, Clarke et al. (2007) found that voles and house mice could be experimentally infected and that voles shed the bacteria in their feces. As a result, they suggested that some rodent species may also serve as spillover hosts at farms in Michigan, USA. They also suggested that these rodents be removed from farms with infected cattle. Currently, the only wildlife species removed during the depopulation process on cattle-infected farms in Michigan, USA, was white-tailed deer and only in some cases.

Environmental contamination of infected farms was not documented in this

study. Additional targeted effort and improved methodologies may be needed to more adequately address this issue and effectively isolate *M. bovis* from environmental samples. In an outdoor study of environmental persistence of bovine TB on various substrates in Michigan, Fine (2006) found that the bacteria remained viable under cool, wet conditions for up to 88 days in soil, 58 days in water and in hay, and 43 days on shelled corn. Palmer et al. (2004) confirmed that white-tailed deer can be infected by consuming feed that had been previously exposed to infected animals. Hence, when depopulating an infected cattle farm, it is probably a prudent practice to remove cattle feed, bedding, and water and to disinfect all surfaces.

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