

Evaluation of Potential Shedding of *Mycobacterium bovis* in Free-Ranging Raccoons

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ABSTRACT: Bovine tuberculosis (bTB) is a contagious disease capable of infecting wildlife, livestock, and humans. While once common in U.S. livestock, the disease has historically been rare in wildlife. However, in Michigan's Northeastern Lower Peninsula (NELP), bTB is endemic in white-tailed deer, and evidence suggests transmission to cattle. The disease has also been documented in other wildlife species including raccoons, which frequent areas used by domestic cattle. Such interactions could facilitate transmission of bTB, but whether free-ranging raccoons shed the causative agent, *Mycobacterium bovis*, is unknown. We trapped raccoons on private and public land in 5 counties in the NELP from which we collected tissue samples, oral/nasal swabs, and fecal samples to determine if raccoons shed *M. bovis*. Culture results from 2 of 144 usable tissue sample submissions were positive for bTB, suggesting an apparent local prevalence of 1.4%, a decrease from previous estimates. Using currently available culturing techniques, swabs and feces from one tissue culture-positive animal were negative for *M. bovis*. While this small sample size of positive animals makes definitive conclusions difficult, we believe that although raccoons may serve as a reservoir or a spillover host for bTB, transmission risk to cattle is minimal. Further research into this arena, as well as continued refinement of culturing techniques to detect low levels of *M. bovis*, is warranted.

KEY WORDS: bovine tuberculosis, Michigan, *Mycobacterium bovis*, *Procyon lotor*, raccoon, wildlife disease

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INTRODUCTION

Bovine tuberculosis (bTB) is a contagious disease caused by the bacterium *Mycobacterium bovis*, capable of infecting wildlife, livestock, and humans. During the early part of the 20th century, bTB was prevalent in U.S. cattle. Since 1917, the Bureau of Animal Industry and later the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service has made major progress in eliminating the disease from domestic livestock. By the mid-1990s, only a few known infected cattle herds remained (Frye 1995); however, the role of wildlife in bTB transmission in the United States remained largely unexplored. In 1994, a hunter-killed white-tailed deer (*Odocoileus virginianus*) was found infected with bTB in Michigan's Northeastern Lower Peninsula (NELP) (Schmitt et al. 1997), and by 1998 the disease had spread to cattle (Schmitt et al. 2002). This resulted in revocation of Michigan's Accredited TB-free status by the USDA (USDA 2000). Currently, Michigan is classified with split-state status, with restrictions on inter and intrastate movement of cattle (Michigan Department of Agriculture 2010). Furthermore, a special Deer Management Unit (DMU 452) was established surrounding the epicenter of the bTB outbreak.

To reduce the potential spread of bTB from deer to cattle, efforts were undertaken to reduce deer densities in DMU 452. Although the apparent prevalence of bTB in deer appears to be declining (O'Brien 2008), infected cattle farms and wild deer are still documented annually.

Questions remain surrounding the possibility of other reservoirs for the disease.

Bruning-Fann et al. (2001) documented several other wildlife species infected with bTB, including black bears (*Ursus americanus*), coyotes (*Canis latrans*), raccoons (*Procyon lotor*), and red fox (*Vulpes vulpes*). All of these species could potentially shed *M. bovis* and pose a risk to livestock. However, primary species of concern for transmission of bTB from wildlife to domestic livestock are those in frequent contact with livestock or areas in which direct or indirect contact may occur, i.e., feed storage sites, livestock feeding locations, etc. Co-use of resources by raccoons and domestic livestock may contribute to the spread of bTB between the species (VerCauteren et al. 2005, Atwood et al. 2009). Research by Palmer et al. (2004a,b) suggests transmission of bTB is possible through shared feed. Limited shedding of *M. bovis* via oral/nasal secretions and feces has been documented in laboratory trials with raccoons and opossums (*Didelphis virginiana*) (Palmer et al. 2002, Fitzgerald et al. 2003). However, the question of *M. bovis* shedding by naturally infected raccoons has not been investigated.

It is essential to determine if species other than white-tailed deer shed *M. bovis* and could serve as a reservoir or transmission host for the dissemination of *M. bovis* to other wildlife or domestic animals. To determine if an animal is infected with bTB, culturing suspected tissues is the gold standard. However, identifying an animal as

infected does not address whether that individual can transmit the disease. This study provides information on whether naturally infected wild raccoons shed *M. bovis* and their potential in the spread of bTB to domestic cattle.

STUDY SITE

Sample collection was performed on 17 currently or previously bTB-infected cattle farms in Alpena, Alcona, Antrim, Montmorency, and Oscoda Counties in Michigan's NELP. Additional samples were collected on public land in Alcona County. The regional topography is dominated by forests of jack pine (*Pinus banksiana*) and white pine (*P. alba*), with ephemeral wetlands consisting largely of white cedar (*Thuja occidentalis*). All collection sites were within Michigan's Modified Accredited Zone (Michigan Department of Agriculture 2005) and most were within the bTB core area: DMU 452 (Figure 1).

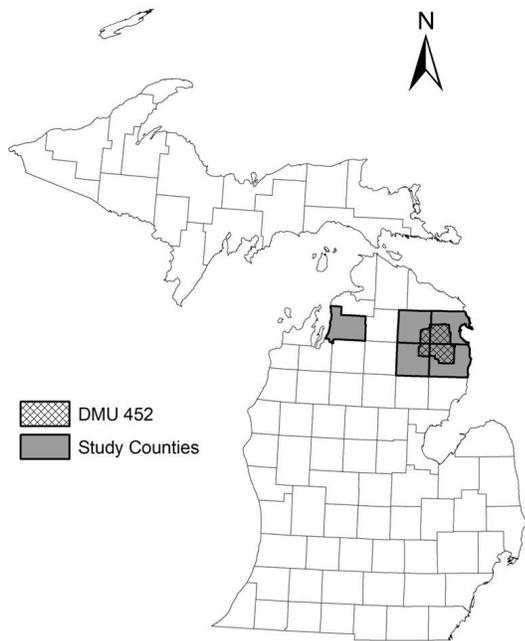


Figure 1. Map of Michigan showing the bTB Modified Accredited Zone and Deer Management Unit 452 in the Northeastern Lower Peninsula.

METHODS

Raccoons were collected by USDA APHIS Wildlife Services wildlife specialists and private landowners in the NELP during routine raccoon removal on currently or previously infected farms from October 2006 through May 2008. Additional samples were obtained by National Wildlife Research Center scientists on public land

from June to July 2007. Free-ranging raccoons were live trapped using cage traps and euthanized by a .22 caliber gunshot through the brain. Samples from lymph nodes (parotid, retropharyngeal, mandibular, mediastinal, bronchial, and mesenteric) and tissues that appeared to contain lesions were obtained. In addition, two oral/nasal swabs and fecal samples (when available) were collected for culture to determine shedding potential. Samples were obtained either immediately in the field or carcasses were frozen and shipped to the USDA Wildlife Services laboratory in Lansing, MI, where necropsies and sample collection were performed. Tissue samples were pooled under head, thorax, or abdomen categories. One-half of the samples from each category were frozen, and the other half stored in formalin. Tissue samples were shipped to the USDA National Veterinary Services Laboratories (NVSL) in Ames, IA, for histological analysis and culture following established protocols (NVSL 2006). Swabs and fecal samples from animals whose tissues were positive on culture at NVSL were sent to the Colorado State University Department of Microbiology, Immunology, and Pathology for culturing using procedures modified from Whitlock and Rosenberger (1994).

RESULTS

One hundred fifty-three raccoons were trapped from five counties from 2006 to 2008 (Table 1). Fourteen were obtained on public land and 139 from privately-owned farms previously infected with bTB. Tissue samples from 151 raccoons were submitted for culture. Seven tissue samples negative under gross histopathology were contaminated and unusable for culture. One sample (Alcona County) cultured positive for *M. bovis*. An additional sample (Alpena County) cultured positive for the *Mycobacterium tuberculosis* complex, which includes *M. bovis*, but contamination precluded speciation. This sample is assumed to be positive for *M. bovis* due to the low likelihood of a raccoon being positive for *M. tuberculosis*, the human form of TB. Both samples were obtained from raccoons on previously infected farms. Swabs and fecal samples from one of the bTB positive raccoons cultured negative for *M. bovis*. Swabs and fecal samples from the second bTB positive raccoon were never received by the CSU laboratory, resulting in no culturing.

DISCUSSION

Prevalence estimates of bTB in raccoons in the NELP range from 2.5 to 4.7% (Bruning-Fann et al. 2001, Witmer 2006). Our study resulted in an apparent prevalence of 1.4%, a decrease from previous reports. If raccoons were shedding the causative agent of bTB, it seems logical that the apparent prevalence would be higher due to horizontal transmission between raccoons. In addition, given the activity of raccoons in feed storage

Table 1. Number of raccoons trapped by county and year in Michigan's NELP, 2006-2008.

Year/County	Alcona	Alpena	Antrim	Montmorency	Oscoda
2006	0	16	0	4	0
2007	19	32	14	25	0
2008	22	9	0	8	4
Total	41	57	14	37	4

areas, it follows that if raccoons were shedding, we would see an increase in bTB positive cattle farms as well, but neither of these scenarios has appeared. However, it must be taken into consideration that the farms on which raccoons were collected had undergone annual raccoon removal for multiple years prior to this study. It is possible these sites were “trapped-out” of positive animals. If this is the case, it suggests that trapping the same property for multiple years may reduce the prevalence of bTB-positive raccoons.

The fact that swabs and fecal samples from the positive raccoon, albeit only one, cultured positive suggests that raccoons, while a potential reservoir for bTB, may not pose a significant transmission risk. Indeed, Palmer et al. (2002) found that only intravenous inoculation or multiple oral doses of *M. bovis* induced shedding in raccoons. Fitzgerald et al. (2003) documented similar results in opossums. Furthermore, Johnson et al. (2008) and Berentsen et al. (2010) failed to document shedding of *M. bovis* in either orally inoculated captive or bTB-positive free-ranging coyotes. Whether doses experimentally administered to raccoons are comparable to actual concentrations of *M. bovis* raccoons would be exposed to under natural settings remains unknown.

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