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Abstract: Bovine tuberculosis (bTB) is a zoonotic disease caused by *Mycobaterium bovis*, and is transmissible to humans, wildlife and domestic livestock. In the northern Lower Peninsula of Michigan, white-tailed deer (*Odocoileus virginianus*) serve as a reservoir for the disease and pose a significant threat to domestic cattle and captive cervids. Scientists at USDA, APHIS, Wildlife Services, National Wildlife Research Center have designed a variety of laboratory and field studies aimed at reducing or eliminating bTB infection in cattle by interrupting the transmission of the disease from wildlife reservoirs. These strategies include reducing bTB in deer by delivery of oral vaccines, creating effective barriers to cattle/wildlife interactions, determining the role of other wildlife species in the transmission of *M. bovis*, and evaluating a sentinel species to monitor prevalence of bTB in the environment. In addition, studies are being conducted to assess transmission risks between deer and cattle on cattle farms and to recommend mitigation measures to reduce these risks. These studies will provide comprehensive information on the role of wildlife-livestock interactions in the maintenance and spread of bTB as well as recommendations of measures to contribute toward its eventual eradication in domestic cattle in cases where wildlife is the source of infection.

***Key words*:** bovine tuberculosis, domestic cattle, Michigan, *Mycobacterium bovis*, *Odocoileus virginianus*, white-tailed deer, wildlife diseases

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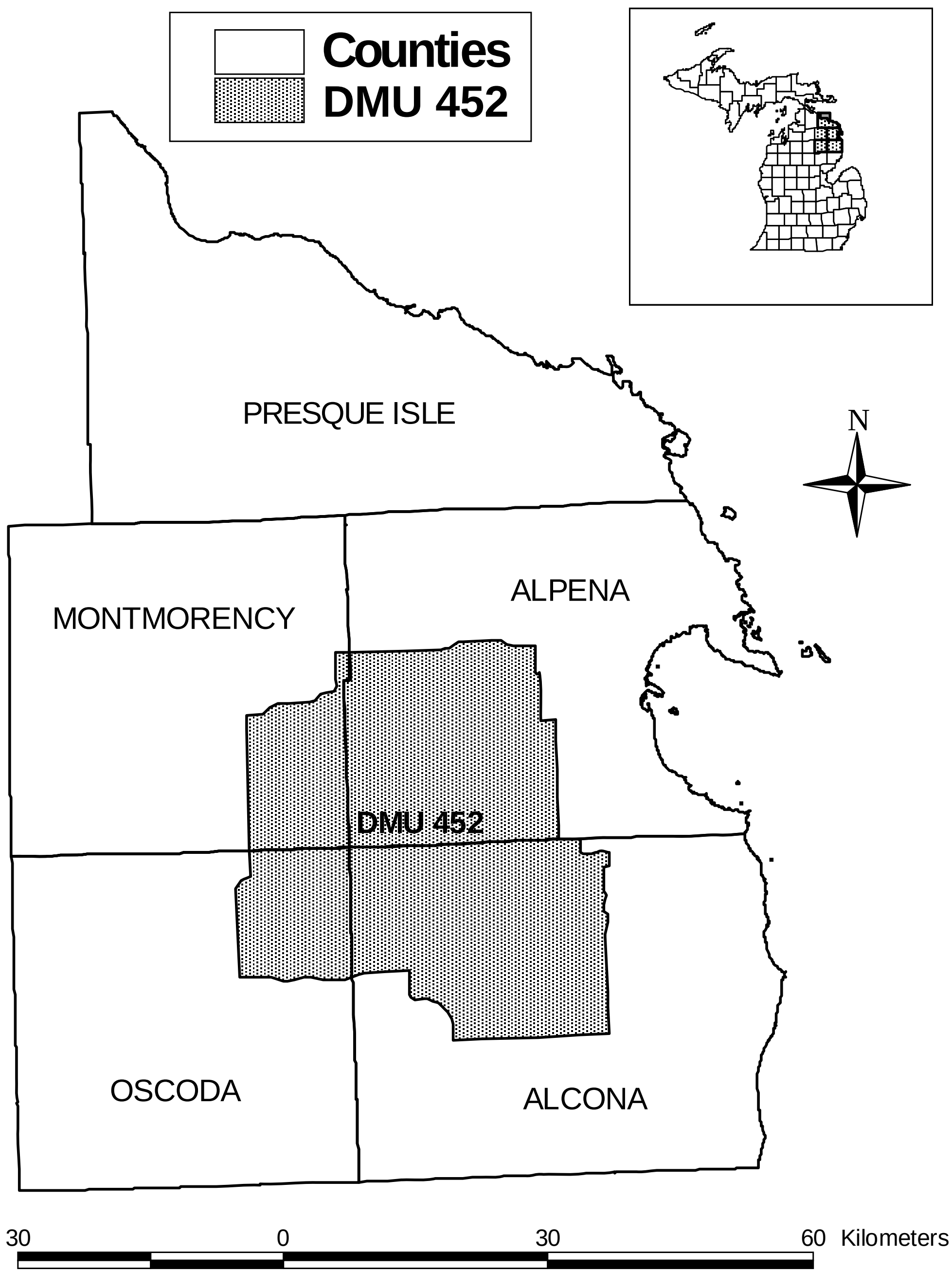
INTRODUCTION

Bovine tuberculosis (bTB) is a contagious disease of both animals and humans. It is caused by the bacterium *Mycobacterium bovis* which is closely related to *M. tuberculosis*, the most common cause of human tuberculosis. *Mycobacterium bovis* can infect a wide range of animals and is a threat to livestock, wildlife, and human health throughout the world. Although bTB was once relatively common in cattle in the United States, it has historically been rare in wildlife. In 1975, a hunter-killed white-tailed deer (*Odocoileus virginianus*) from Michigan's northeastern Lower Peninsula (NELP) was diagnosed with bTB (Stuht and Fay 1976). Subsequent field investigations revealed no additional cases and nearby cattle were not tested (O'Brien et al. 2006). In 1979, Michigan was declared tuberculosis free by the United States Department of Agriculture (USDA). However, in 1994 another bTB positive hunter-killed white-tailed deer was found in the NELP, approximately 13 km from the 1975 case (Schmitt et al. 1997) and evidence suggests that deer can transmit the disease to cattle (Palmer et al. 2004a). Discovery of bTB in domestic cattle resulted in USDA revoking Michigan's Accredited TB-Free status in 1998. Michigan currently has split-state TB-free accreditation status with no bTB reported in the Upper Peninsula.

Figure 1. The five-county bTB outbreak area and DMU 452 in Michigan's Northeastern Lower Peninsula (adapted from Hill 2005).



Bovine tuberculosis in Michigan is most prevalent in a five-county area with Deer Management Unit (DMU) 452 at its core (Figure 1). A number of measures have been implemented in an interagency effort to prevent the spread of the disease and decrease local prevalence in livestock and deer. These include statewide testing of cattle herds and hunter-killed deer, and depopulation of infected cattle and captive deer herds. In addition, Michigan Department of Natural Resources (MDNR) has applied liberal hunting seasons to reduce deer densities, and restrictions on artificial feeding of deer to avoid concentrating deer in areas where disease transmission is most likely to occur (Schmitt et al. 2002). These actions have reduced the average prevalence of bTB in deer in the affected area from 4.9% to 1.7% since 1995 (MDNR 2005). Despite these efforts, between 1997 and 2004, 33 cattle herds and one captive deer herd were infected with bTB (MDNR 2005), presumably through contact with wildlife. Furthermore, an additional six cattle farms and one captive deer farm tested positive in 2006 (B. Nelson, Michigan Department of Agriculture, personal communication). Without further reductions in deer density and other measures to reduce local deer concentrations, the disease may remain enzootic, and continue to pose a risk to Michigan's cattle industry. In addition, other wildlife species may harbor *M. bovis* and frequent farms, possibly spreading the bacterium to cattle. Livestock and deer can contract bTB from saliva or airway secretions transferred directly between animals, or indirectly from eating contaminated feed (Palmer et al. 2004a,b). Therefore, additional measures must be sought to further prevent contact, either direct or indirect, between livestock and wildlife.

Scientists at the United States Department of Agriculture (USDA) Animal Plant and Health Inspection Service (APHIS) Wildlife Services (WS) National Wildlife Research Center (NWRC) are conducting research around several strategies aimed at reducing transmission of bTB from wildlife to cattle. These studies are in varying stages of completion and are part of the NWRC's multi-year project to investigate bTB at the wildlife/livestock interface. Our research falls into four categories outlined in this paper: 1) developing and testing of baits and vaccines; 2) creating barriers to wildlife/cattle interactions; 3) determining the role of wildlife species in the transmission of bTB, and 4) evaluating the use of sentinel species to monitor prevalence of bTB in the environment.

DEVELOPMENT AND TESTING OF BAITS AND VACCINES

The development of effective oral vaccine-laden bait could significantly aid the management of bTB in Michigan. The NWRC is collaborating with several agencies including USDA, APHIS, Veterinary Services (VS), USDA, Agriculture Research Services (ARS) National Animal Disease Center (NADC), Colorado State University (CSU), and AgResearch and the University of Otago in New Zealand, to pursue orally deliverable vaccine bait for bTB in white-tailed deer. A lipid-formulated bait containing the human vaccine *M. bovis* bacille Calmette-Guerin (BCG) has been shown to be effective in mice (*Mus* sp.) (Aldwell et al. 2003a), brushtail possums (*Trichosurus vulpecula*), and cattle in New Zealand (Aldwell et al. 2003b, Buddle et al. 2005). In 2005, vaccine baits were tested in captive white-tailed deer at NADC. Two groups of yearling does were fed lipid baits containing

BCG or were given BCG in liquid media directly to the back of the mouth. Another group received vaccine subcutaneously and a fourth group received placebo and served as control. Several months after challenge with *M. bovis* the control subjects had developed overall significantly more lesions than did the vaccinates (P. Nol, NWRC, unpublished data).

The next step in this process is to develop baits palatable to white-tailed deer that can accommodate the BCG vaccine without compromising its viability and immunogenic properties. Another criterion is that the bait must be deliverable either by aircraft or bait stations. Once the bait has been developed, a field evaluation will take place, possibly as early as 2008.

CREATING BARRIERS TO WILDLIFE/CATTLE INTERACTIONS

The primary method of bTB infection is through inhalation of contaminated aerosols or fomites (Kaneene and Pfeiffer 2006). However, direct contact and sharing of feed between infected and non-infected animals (Palmer et al. 2004a,b) and direct fence line contact (VerCauteren, et al. 2007) have also been implicated in transmission of disease. This suggests that direct and indirect interaction between cattle and deer can pose a significant bTB risk. To decrease the potential for deer-cattle interactions, particularly in feeding areas, NWRC scientists have studied a variety of psychological, physical and biological barriers. Frightening devices (Seward et al. 2007), laser lights (VerCauteren et al. 2006a), propane gas exploders (Gilsdorf et al. 2004) and fencing (VerCauteren et al. 2006b, Seamans and VerCauteren 2006), have all been evaluated with varying efficacy in deterring deer from crops and cattle feed. Livestock guarding dogs have long been used to protect domestic sheep from predators (Green and Woodruff 1990, Andelt 1992), and this concept has been expanded by NWRC scientists to keep deer away from livestock and agricultural crops and reduce the potential for disease transmission (VerCauteren, NWRC, unpublished data). In addition, USDA, APHIS, Wildlife Services in Michigan has conducted extensive programs to install exclusionary fencing around feed storage areas on cattle farms in and around the bTB infected area (MDNR 2006).

DETERMINING THE ROLE OF WILDLIFE SPECIES IN THE TRANSMISSION OF TB

To estimate the apparent prevalence of bTB infected deer in Michigan, MDNR tested 141,550 white-tailed deer and found 509 positive cases over a ten-year period (MDNR 2005). However, white-tailed deer are not the only species of wildlife in Michigan to contract bTB. To evaluate which additional species may be positive for the disease MDNR also tested elk (*Cervus elaphus*), moose (*Alces alces*) and 16 carnivore/omnivore species (Bruning-Fann et al. 1998, 2001). Overall, samples from elk, coyotes (*Canis latrans*), raccoons (*Procyon lotor*), black bears (*Ursus americanus*), bobcats (*Felis rufus*), opossums (*Didelphis marsupialis*), and red foxes (*Vulpes vulpes*) tested positive for bTB (Bruning-Fann et al. 1998, 2001). In addition, NWRC scientists collected 1,039 specimens from 32 species and opossums, raccoons and gray fox (*Urocyon cinereoargenteus*) tested positive for *M. bovis* in these trials (Witmer, NWRC, unpublished data). Research by MDNR and NWRC suggests that although a relatively high prevalence of bTB is found in white-tailed deer compared with other species, other species cannot be disregarded as potential reservoirs.

NWRC scientists have designed field and laboratory studies to determine whether coyotes and raccoons actively shed *M. Bovis* through feces and oral/nasal secretions. We are collecting tissue, fecal and oral/nasal samples from free ranging coyotes from infected counties, and testing them for the presence of *M. bovis*. Fecal and oral/nasal samples from subjects testing positive in tissue samples will be analyzed for the presence of bTB using highly modified Polymerase Chain Reaction (PCR) methods, initially developed for use in deer by scientists of the Animal Population Health Institute at CSU, to determine whether the bacterium is being shed (Triantis et al, unpublished data). Preliminary results suggest that the bTB strain found in Michigan coyotes, cattle, and white-tailed deer are identical to each other, but different from strains found elsewhere in the United States (R. Jones, CSU, unpublished data).

In the laboratory captive coyotes have been inoculated with *M. bovis* cultures. Feces and oral/nasal secretions will be collected and screened for the bacterium using the same culturing and PCR procedures. Because of their relatively high sensitivity to *M. bovis,* laboratory guinea pigs (*Cavia porcellus*) will be exposed to feces from inoculated coyotes to determine whether transmission of bTB can occur through exposure to infected feces.

Coyotes and raccoons are two species that frequently visit livestock operations. Indeed, it would be difficult to find a barn or feed storage area that does not harbor a family of raccoons. Furthermore, coyotes may be frequent visitors to pastures and deposit potentially infected feces while patrolling territorial boundaries or looking for prey. If we are able to determine that either of these species actively shed *M. bovis*, we may be able to recommend mitigating measures to decrease potential contact between these potentially infected species and domestic cattle. If PCR techniques prove valuable in detecting *M. bovis* in feces, studies will be initiated to conduct surveillance of shedding in other small mammals that regularly have contact with cattle feeding operations.

In January 2007, NWRC initiated a study in the NELP to evaluate deer movements in relation to farms in order to determine risk factors associated with transmission of bTB. Guidelines concerning a variety of risk factors have been developed (Kaneene et al. 2002); however, we are attempting to provide a finer scale resolution to the matter by analyzing deer movements in relation to feeding areas, feeding schedules, pastures, fences, food storage facilities, etc. Farms were selected based on several criteria, including similar acreage, cattle present throughout the year, adequate deer habitat, and previous participation in the Wildlife Services Fencing Program. Since January 2007, 17 deer on 5 farms were captured and fitted with GPS collars programmed to collect daily locations every two hours for one year, with trapping to continue through May 2007. Upon retrieving the collars and downloading the data in early 2008, we will evaluate which areas on cattle farms are frequented by collared deer, and to what extent they interact with cattle management operations, thus posing a risk for spreading bTB. In addition, we will evaluate the number of deer locations collected on verses off cattle farms, and the proportion of individual deer home ranges that occupy cattle farms. This information will assist researchers and Wildlife Services specialists in recommending mitigation measures, or changes in livestock management practices, to decrease the risk of bTB in their herds as a result of contact with potentially infected deer.

EVALUATING THE USE OF SENTINEL SPECIES TO MONITOR PREVALENCE OF bTB IN THE ENVIRONMENT

To evaluate whether coyotes can be used as a sentinel species, NWRC scientists are sampling coyotes from counties surrounding the five-county outbreak area. A 5-km buffer zone has been established to minimize the potential of sampling within home ranges of coyotes from infected counties. Tissues, oral/nasal swabs, and fecal samples will be analyzed for the presence of *M. bovis* using PCR and culturing as described above. A recent study found that 24% of coyotes tested in the Michigan bTB affected area were positive for *M. bovis*. This relatively high prevalence in coyotes would enable managers to sample 80% fewer animals and increase detection by 150% (VerCauteren et al., unpublished data) over sampling deer alone. The ability to use coyotes as sentinels to detect bTB in uninfected areas can provide a useful tool for early detection of the disease, particularly in the Modified Accredited Advanced and TB-Free Zones, where annual whole-herd testing is not required.

CONCLUSION

Intensive management actions by several agencies have dramatically reduced the apparent prevalence of bTB in the outbreak area of Michigan. However, it is still too early to claim victory over the disease. Domestic cattle herds are still at risk of infection and eradication of bTB from wild deer has not yet been achieved, and the disease is likely to persist for at least another decade (O'Brien et al. 2006). Our multi-faceted research strategy, coupled with continued monitoring and management of wildlife reservoirs by MDNR is another vital step toward managing and eventually eliminating bTB in Michigan.

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