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LIMITED ANTIBODY EVIDENCE OF EXPOSURE TO *MYCOBACTERIUM BOVIS* IN FERAL SWINE (*SUS SCROFA*) IN THE USA

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ABSTRACT: Bovine tuberculosis is a chronic disease of cattle (*Bos taurus*) caused by the bacterium *Mycobacterium bovis*. Efforts have been made in the US to eradicate the disease in cattle, but spillover into wildlife and subsequent spillback have impeded progress in some states. In particular, infection in white-tailed deer (*Odocoileus virginianus*) has been followed by infection in cattle in some Midwestern states. Infection has also been documented in feral swine (*Sus scrofa*) on the Hawaiian island of Molokai and in various European countries, but no large-scale survey of antibody exposure to the bacteria has been conducted in feral swine in the US. We tested 488 sera from feral swine collected near previously documented outbreaks of bovine tuberculosis in cattle and captive cervids, in addition to 2,237 feral swine sera collected across the US from 1 October 2013 to 30 September 2014. While all but one of the samples were antibody negative, the results are important for establishing baseline negative data since feral swine are capable reservoirs and could be implicated in future outbreaks of the disease.

Key words: Bovine tuberculosis, feral swine, *Mycobacterium bovis*, *Sus scrofa*, tuberculosis, wild pig.

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic bacterial disease of cattle (*Bos taurus*), caused by *Mycobacterium bovis*, which occasionally affects other mammals. Clinical signs may include emaciation, lethargy, weakness, and respiratory problems and are typically not detected until later stages of disease progression. Though BTB is not considered a threat to commercial swine in the US, it is important to both the cattle and captive cervid industries (Witmer et al. 2003).

In 1917, BTB was recognized as a significant disease of livestock in the US, and a national cooperative state-federal BTB program was established to address the issue. This program was motivated by a desire to reduce the number of infected carcasses reaching the kill floor at slaughter facilities, and thus entering the human food chain (Essey and Koller 1994). Since the development of the program, the US has established tuberculosis-free status in all states except California and Michigan (US Department of Agriculture's Animal and Plant Health Inspection Service [USDA-APHIS] 2016). Until states obtain tuberculosis-free status, they are required to conduct additional cattle testing and are banned from exporting cattle to other states or countries, which impacts them financially.

In North America, *M. bovis* has been identified in 10 wildlife populations and remains endemic in white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk

(*Cervus elaphus nelsoni*), and American bison (*Bison bison*; Miller and Sweeney 2013). In three of the 10 wildlife populations in North America (Michigan, Manitoba, and Alberta) known to currently have endemic *M. bovis* infection, all are believed to become established via initial transmission of *M. bovis* from cattle to wildlife (Miller and Sweeney 2013). Once established in wildlife populations, a continued risk persists for reinfection of cattle, complicating eradication efforts. While cervid species such as white-tailed deer are perhaps the most well-known wildlife reservoir for the bacteria in the US, infection has been documented in additional species. One of these species is feral swine (*Sus scrofa*), a prolific and invasive species that is highly adaptable and has greatly expanded its geographic range over the past few decades (Bevins et al. 2014). In the US, feral swine are defined as escaped or intentionally released domestic swine, wild boar, or hybrids of the two (Mayer and Brisbin 2008).

Although it has been established that feral swine can serve as reservoirs of BTB (Naranjo et al. 2008), the disease has been reported only once previously in feral swine in the continental US. In this case, feral swine were sampled in California from 1965 to 1968 as part of control efforts on an *M. bovis*–infected beef cattle farm, and 11.9% were culture-positive (Smith 1968). A more recent study along the southern border of Texas was conducted in 2010, but all 396 feral swine sampled were culture-negative (Campbell et al. 2011). A population of feral swine infected with *M. bovis* exists on the Hawaiian island of Molokai (Essey et al. 1981). After an infected cattle herd was removed from the area, the prevalence of BTB in feral swine declined dramatically from 20% to 3.2% (Essey et al. 1983). Initially, it was suggested that the disease was maintained on the island through spillover from cattle; however, *M. bovis* was detected in feral swine after the removal of cattle indicating that the bacteria could persist without cattle. *Mycobacterium bovis* infection also has been reported in native Eurasian wild boar in Spain (Naranjo et al. 2008), Portugal (Duarte et al. 2007), Italy (Pavlik et al. 2002), France (Richomme et al. 2013), Croatia, Hungary, Slovakia, and Russia (Machackova et al. 2003), the United Kingdom (Foyle et al. 2010), Germany (Schulz et al. 1992), and Morocco (El Mrini et al. 2016), and in feral swine in Australia (Corner et al. 1981) and New Zealand (Wakelin and Churchman 1991). *Mycobacterium bovis* is considered endemic in wild boar and feral swine in the US (Hawaiian island of Molokai), France, Italy, Portugal, and Spain with prevalence rates ranging from 1.4% to 92%. Seven countries (US, Australia, New Zealand, Spain, Portugal, France, and the UK) have documented transmission between cattle and wild boar or feral swine (Corner et al. 1981; Essey et al. 1981; Wakelin and Churchman 1991; Foyle et al. 2010; Richomme et al. 2013).

While there have been no reports of BTB in feral swine in the continental US since the initial report in the 1960s, no national-level surveys have been conducted to confirm its absence. To better understand the role of feral swine in the persistence of BTB in the US, we tested sera collected from feral swine in counties where outbreaks had occurred in livestock previously, as well as sera collected elsewhere across the US. Our objective was to determine whether feral swine in the US have been exposed to *M. bovis*, and to establish baseline data for potential future outbreaks of the disease.

MATERIALS AND METHODS

Sample collection

The USDA-APHIS Wildlife Services routinely collects serum samples from feral swine removed for wildlife damage management purposes. Serum from each animal is tested immediately for exposure to pathogens and is archived for future testing. From the archive, we selected sera collected from feral swine from 2006 to 2012 in the same counties or states (if no samples were available from the county) where outbreaks had occurred previously in cattle and captive cervids (USDA-APHIS 2016). In addition, all feral swine sera collected by USDA APHIS Wildlife Services from 1 October 2013 through 30 September 2014 were tested to establish baseline data for feral swine exposure to BTB in the US (Fig. 1).

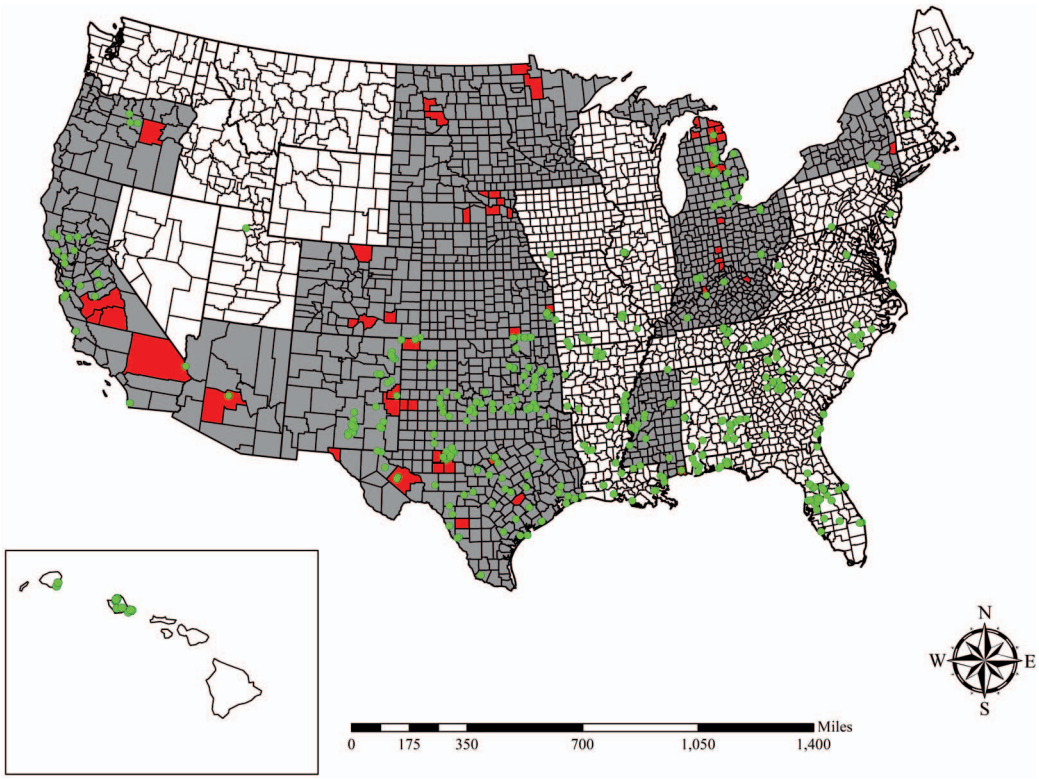

FIGURE 1. Locations in the US where feral swine (*Sus scrofa*) serum samples that were tested for exposure to bovine tuberculosis were collected (green dots) from 2007 to 2015. Sampling locations are overlaid with the counties (red) and states (gray) where outbreaks in domestic livestock have been reported since 1997.

Serology

Feral swine sera were screened with an indirect enzyme-linked immunosorbent assay (ELISA) using *M. bovis*–purified protein derivative (obtained from the National Veterinary Services Laboratories, Ames, Iowa, USA) as the antigen, along with horseradish peroxidase-conjugated protein G to test for anti-purified protein derivative immunoglobulins. Serological positive and negative controls were obtained from wild boar in Spain previously identified as *M. bovis* culture-positive or culture-negative.

The ELISA was conducted following previously described methods with slight modifications (Boadella et al. 2011). Briefly, antigen-coated plates were incubated at room temperature for 18 h, followed by one wash with phosphate-buffered saline with 0.05% Tween 20 (PBST; Sigma-Aldrich, St. Louis, Missouri, USA) prior to blocking with 5% skim milk in PBST. Sera were diluted 1:20 in phosphate-buffered saline, and then further diluted 1:10 in blocking solution in each well of the Nunc Maxisorp ELISA plate (Sigma-Aldrich). The plate was incubated for 60 min at room temperature (with no shaking). Plates were manually washed three times with 300 µL per well of PBST prior to the addition of 100 µL per well of protein G diluted to 0.5 µg/mL, followed by a 90 min incubation at room temperature with no shaking. The plates were washed three more times with 300 µL per well of PBST, followed by the addition of SigmaFast OPD developing solution (Sigma-Aldrich), and then incubated for 20 min at room temperature. After incubation, 50 µL per well of 3N sulfuric acid was used to stop development, and the plate was read at 450 nm to calculate the sample optical density (OD). All samples were tested in duplicate. The ELISA values were calculated using the formula: mean sample OD/2 × mean negative control OD × 100, and values greater than 1 were considered positive (Boadella et al. 2011).

Determination of serological prevalence

We used a Bayesian model (Messam et al. 2008) to estimate national-level prevalence and probability of freedom of *M. bovis* in feral swine. Samples collected in the same county were

TABLE 1. Number of feral swine (*Sus scrofa*) serum samples by age class and gender collected in the US from 1 October 2013 through 30 September 2014, and tested for exposure to bovine tuberculosis with an indirect enzyme-linked immunosorbent assay.

	Gender		
Age class	Male	Female	Unknown
Adult	844	965	8
Sub-Adult	321	292	2
Juvenile	137	132	1
Unknown	3	3	17

assumed to originate from the same feral swine population, and samples collected in the same month and year were considered a single sampling event. The ELISA used for detection has an estimated sensitivity (SN) of 79.2% and a specificity (SP) of 100% (Boadella et al. 2011). However, there was uncertainty regarding the test performance for feral swine and *M. bovis* in the US because the serological controls originated from wild boar in Spain. This uncertainty was accounted for by using beta-distributed priors for SN (α=56.1, β=15.5) and SP (α=28.9, β=1.03) assuming 95% certainty that the ELISA SN was greater than 70% and SP was greater than 90%. We assumed no prior knowledge of prevalence using a vague beta distribution (α=1, β=1). The model was fit using Markov chain Monte Carlo techniques and implemented in R (R Core Team 2012) and JAGS software (Plummer 2013). Posterior inference used 100,000 iterations with the first 20,000 iterations discarded. Convergence was confirmed by using autocorrelation among samples and the Brooks-Gelman-Rubin convergence statistic (Gelman et al. 2014). We used the highest posterior density (HPD) as an estimate of the expected national prevalence given the animals sampled. To estimate probability of disease freedom we used the World Organisation for Animal Health (OIE) threshold of 0.1% for animal level prevalence (OIE 2016) and evaluated if the upper 95% HPD credible interval was greater than this threshold. We also calculated the probability that the prevalence was below this threshold.

RESULTS

Sera from 2,725 feral swine collected from 2007 to 2015 in 233 counties of 31 states were tested for exposure to bovine tuberculosis (Fig. 1). This resulted in 902 sampling events with samples per event ranging from 1 to 44. The age class and gender of the feral swine tested was approximately equal between sexes (Table 1).

One feral swine sampled at Woody Bayou in Hancock County, Mississippi, in July 2014 tested positive on initial testing (ELISA value=1.4) and positive on two additional repeats of the test (ELISA values=1.5 and 1.4), resulting in a 94% chance the result was a true positive. Subsequently, additional archived feral swine sera from Hancock County were tested, but all samples were antibody negative (n=96), and using the Bayesian model there was a 95% chance the prevalence was 0.00003% (HPD 95% credible interval=7.3×10^{-9} to 4.1×10^{-3}%). All other samples tested antibody negative.

Based on our results, the estimated national HPD for prevalence was 0.00003%, and there is a 95% chance that the estimated prevalence of *M. bovis* in feral swine in the US was <0.0037% and a 99% chance that it was <0.0057%. There was >99% chance the estimated prevalence was below the 0.1% threshold for disease freedom established by OIE (2016).

DISCUSSION

Even though there have not been any previous outbreaks of BTB in cattle or wildlife in Hancock County, Mississippi, BTB has been reported in other parts of the state previously. In 2007, a BTB-infected rodeo bucking bull was identified at slaughter (though BTB was not confirmed in the herd of origin), and in 2010, a roping steer was identified as positive, and subsequent trace-back investigations led to identification of an additional positive beef herd (Portacci et al. 2014). Despite the known presence of BTB in Mississippi, it is also possible that the positive feral swine result was a false positive caused by a cross-reaction with another species of *Mycobacterium* or another organism. Anthropogenic movement of feral swine has been suggested (Sweitzer et al. 2015) and could be yet another explanation for the single positive

detection. Additionally, the ELISA had a moderate sensitivity of 79.2%, which may have resulted in false negatives in the sera tested. Regardless, additional monitoring of domestic livestock and wildlife in this geographic area is recommended.

Infection with BTB has been documented previously in feral swine on the Hawaiian island of Molokai (Essey et al. 1981). Although none of the serum samples available for our testing were collected on Molokai, 121 feral swine collected in Honolulu and Kauai counties all tested negative. This supports more recent assessments that *M. bovis* infection in feral swine is isolated to Molokai Island (USDA-APHIS 2006), but additional research would be needed to confirm that.

Infection with *M. bovis* has been shown to persist in wildlife populations for many decades without detection. Examples include Michigan, where *M. bovis* is thought to have persisted for approximately 50 yr before identification, Hawaii's island of Molokai, where *M. bovis* persisted for years among feral swine before being identified in cattle, and Riding Mountain National Park, Manitoba, Canada, where BTB was first identified in 1937 but reemerged in cattle and elk in 1991 more than 50 yr after first being discovered in wildlife (Carbyn 1982). Due to the latency of infection and the potential for ongoing transmission within feral swine populations, we tested 153 samples from San Luis Obispo County, California, where the 1965 outbreak of *M. bovis* occurred in both cattle and feral swine (Smith 1968). We also tested an additional 204 samples from other locations in California (357 total), but all tested negative, indicating that the control efforts during the 1965–68 outbreak where 331 feral swine were removed from the outbreak area (Smith 1968) were likely successful in eliminating BTB from feral swine.

Feral swine contact with cattle and domestic swine is documented (Wyckoff et al. 2009; Cooper et al. 2010) and poses a risk of transmission (Miller et al. 2013). Commingling of cattle and wildlife is associated with the introduction of several pathogens into North American wildlife populations including the introduction of *M. bovis* into white-tailed deer in Michigan (Miller and Sweeney 2013). Several strains of *M. bovis* continue to circulate in North American cattle and domestic cervid populations pose a continued risk of transmission to wildlife (Tsao et al. 2014). In several Mediterranean populations, the European wild boar is a maintenance host for *M. bovis* (Hermoso de Mendoza et al. 2006). In the US, feral swine frequently commingle with cattle across much of their range, posing a continued risk for establishing a new wildlife reservoir for BTB in the US. Due to the highly adaptable nature of feral swine to a wide variety of habitats, as well as their ability to carry and transmit numerous pathogens that affect livestock, this possibility warrants further investigation since it is likely that their impact would be greater than that posed by cervid species. Studies to examine potential contact and transmission between feral swine and cattle are recommended, especially in areas where feral swine populations overlap with outbreaks of BTB in domestic livestock.

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