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ABSTRACT: Although relatively small, Michigan's elk (*Cervus elaphus nelsoni*) herd is highly valued by both hunters and the general public. Elk and red deer (*Cervus elaphus elaphus*) are highly susceptible to infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB), and outbreaks have been documented worldwide. The Michigan elk range lies entirely within counties where TB is known to be enzootic in white-tailed deer (*Odocoileus virginianus*). Consequently, a project was undertaken to estimate the true prevalence of TB in Michigan's free-ranging elk herd. All elk harvested by licensed hunters during 2002–2004, and all nonharvest elk mortalities examined by the Michigan Department of Natural Resources Wildlife Disease Laboratory from November 2002–May 2005, were screened for gross lesions of TB with samples of cranial lymph nodes and palatine tonsils collected for histopathology and mycobacterial culture. In all, 334 elk were included in the study. Twenty-three elk with gross lesions were considered TB suspects; all were culture-negative for *M. bovis*. However, *M. bovis* was cultured from two elk without gross lesions. The sensitivity, specificity, and negative predictive value of the current TB surveillance protocol were 0%, 100%, and 99.4%, respectively, while the apparent prevalence and true prevalence calculated directly from the sample were 0% and 0.6%, respectively. The positive predictive value and the estimated true prevalence of the population were undefined. The poor sensitivity of current surveillance was likely an artifact of its application to a relatively small sample, in order to detect a disease present at very low prevalence. The low prevalence of TB in Michigan elk, and the early stage of pathogenesis of the few infected animals, does not suggest elk are maintenance hosts at the present time.

Key words: Bovine tuberculosis, *Cervus elaphus* spp., diagnostic tests, elk, *Mycobacterium bovis*.

INTRODUCTION

Prior to European settlement, elk (*Cervus elaphus canadensis* Erxleben) were abundant and found throughout the Lower Peninsula of Michigan. Elk were extirpated from the state before 1880 (Burt, 1946). Several attempts to reintroduce elk into the northern Lower Peninsula were made in the early 1900s (Stephenson, 1942). It is generally believed that a release of seven animals in 1918 was successful in re-establishing the species in the state, although genetic evidence suggests other re-introductions (that were presumed to have failed) also contributed (Glenn and Smith,

1993). By 1939, the herd had grown to approximately 400 animals that ranged over 360 km^2 (Shapton, 1940) and by 1960, approximately 1,000 elk were distributed over 1,000 km^2 (Moran, 1973). Limited range to accommodate the increasing elk population resulted in public complaints of damage to agricultural crops and forest regeneration, and in response, an elk management program was established by the Michigan Department of Natural Resources (MDNR) in 1962. Elk hunts were initially held in 1964 and 1965. Annual hunts began in 1984, with the goal of maintaining the population between 800 and 900 animals. The post-harvest population was estimated at 905 elk (95%

confidence limits: 660, 1,150) in late winter 2006 (Walsh, 2007).

While Michigan's elk population is relatively small, compared to herds in the western US and Canada, it is nevertheless highly valued by both hunters and the general public (Bender, 1992). Elk are the premier big game species in Michigan. Since the modern era of elk hunting began in 1984, an average (±SD) of 40,644±4,912 hunters applied for available elk licenses (range: 50–420) each year (MDNR, 2002; MDNR unpubl. data). In addition to hunting, the elk herd is also the object of important nonconsumptive uses such as wildlife viewing (Ryel et al., 1982). Although no Michigan-specific data are currently available, a Pennsylvania study found elk viewing attracted an annual audience of between 60,000 and 75,000 visits per year, generating an estimated annual economic impact of >$2.2 million (Strauss et al., 2001).

Elk, and their congeners eurasian red deer (*Cervus elaphus elaphus*), are highly susceptible to infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB; Clifton-Hadley and Wilesmith, 1991), and outbreaks have been documented in a number of countries worldwide (Griffin and Mackintosh, 2000; table 2). While usually presenting as a localized lymphatic ailment, TB can also be a rapidly spreading fulminating disease, especially in animals exposed to stress (Griffin and Buchan, 1994). Elk have been characterized as the cervid species of greatest epidemiologic concern for bovine TB in North America (Tessaro, 1986) and are both the maintenance host and the primary reservoir in an ongoing outbreak of bovine TB in Manitoba (Lees et al., 2003). Although individuals or small groups can range widely across Michigan's northern Lower Peninsula (Moran, 1973, fig. 6), the primary elk range occupies ~2,500 km^2 (44°52.8′N to 45°22.2′N latitude, 83°55.8′W to 84°46.2′W longitude; MDNR unpubl. data). The southeastern edge of this range is in close proximity to Deer Management Unit (DMU) 452 (O'Brien et al., 2002), the core area of Michigan's bovine TB outbreak in white-tailed deer (WTD; *Odocoileus virginianus*; Fig. 1), and the entire elk range lies within counties where bovine TB is known or presumed to be enzootic (O'Brien et al., 2006) in WTD. The tendency of elk to congregate seasonally in large groups (Moran, 1973), and their capacity to develop subcutaneous abscesses (Rhyan et al., 1992) and fistulous tracts that drain through the skin (Griffin and Buchan, 1994) when infected with *M. bovis*, have raised concerns that bovine TB could spread rapidly within Michigan's elk herd. The finding of grossly lesioned and culture-positive free-ranging elk in 2000 and 2001 confirmed the presence of bovine TB. Consequently, a project was undertaken to estimate the true prevalence of bovine TB in Michigan's free-ranging elk herd. Beyond determining true prevalence, objectives were to quantify the sensitivity, specificity, and the positive and negative predictive values of the current elk surveillance protocol, and to assess the current reservoir status of the elk herd for bovine TB.

MATERIALS AND METHODS

The study population consisted of all elk harvested by licensed hunters during the 2002–2004 seasons and all nonharvest elk mortalities examined by the MDNR Wildlife Disease Laboratory (WDL) from November 2002 through May 2005. This encompassed all of the free-ranging elk accessions available to the WDL during the period. Testing of hunter-harvested elk for bovine TB has been mandatory since 1998 (Schmitt et al., 2002). In addition, carcasses are examined for disease at mandatory elk check stations, and hunters must flag kill sites with their license number so that gut piles can be examined by MDNR field staff for evidence of bovine TB. Except as noted, methods for this study were identical to those described previously for WTD (O'Brien et al., 2004). Elk heads were disarticulated at the check station and identified with uniquely numbered jaw tags documenting sex, harvest location (township, range, and section), and date, and the name, address, and phone
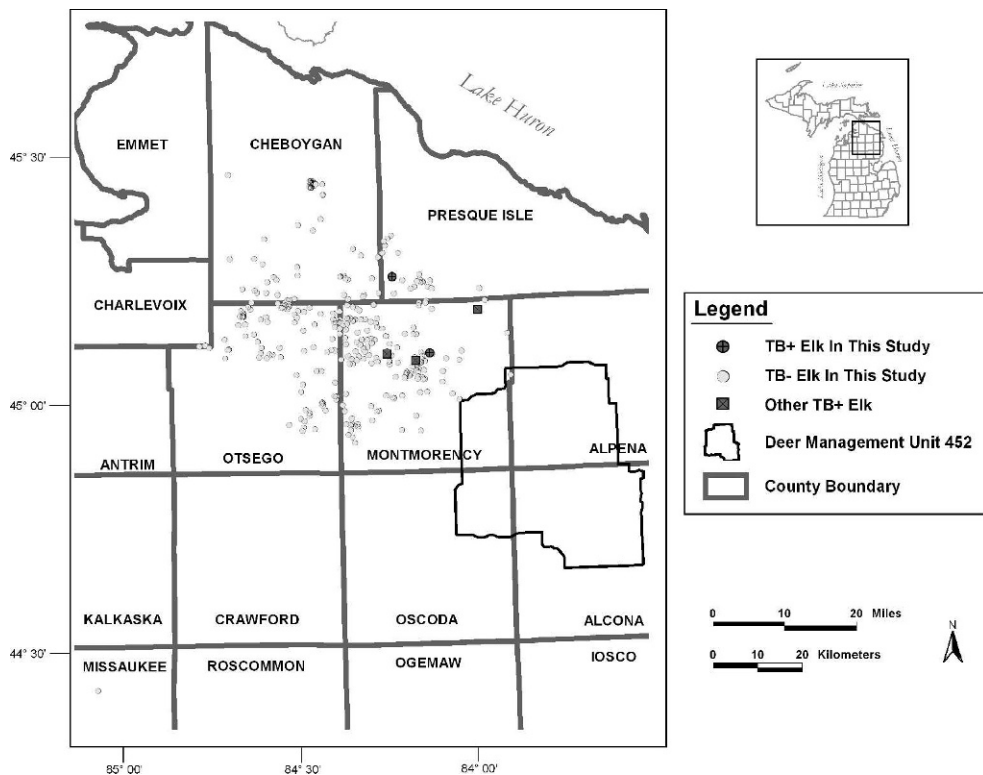
FIGURE 1. Locations at time of death of study elk (round plotting symbols) and other *Mycobacterium bovis*-positive elk found since 2000 (square plotting symbols), in relation to the core outbreak area for white-tailed deer, Deer Management Unit 452.

number of the hunter. Heads were bagged and shipped to the WDL. There, sex was confirmed and age was estimated by tooth eruption and wear (Hudson et al., 2002). All data were archived (Access 2002, Microsoft Corporation, Redmond, Washington, USA). Heads exhibiting cranial lymph node or palatine tonsil enlargement with granuloma formation or gross abscessation were considered suspect, and were diverted into the specimen stream for normal bovine TB surveillance (with tissue sections processed separately for histopathology, acid fast [AF] staining, and bacterial culture). For each head without gross lesions, submandibular, parotid and medial retropharyngeal lymph nodes, and palatine tonsils were dissected bilaterally and pooled, with a portion placed in a sterile, 50-ml polypropylene screw top centrifuge tube (Corning Incorporated, Corning, New York, USA) for bacterial culture; the remainder was placed in 10% neutral buffered formalin (NBF) in a 133-ml plastic screw-top container (VWR Scientific Products, West Chester, Pennsylvania, USA) for histopathology.

All exams were carried out by, or under the supervision of, wildlife veterinarians or pathologists. Necropsy instruments were decontaminated, between heads, by agitation in 70% ethanol sand. Residual ethanol was flamed off, and instruments were cooled momentarily prior to dissection of the next head.

Tissue samples were subjected to mycobacterial testing at the Michigan Department of Community Health (MDCH) Mycobacteriology Laboratory (ML). One- to 2-g sections of each of the collected tissues were combined, ground using individual sterile, disposable tissue grinders (The Kendall Company, Mansfield, Massachusetts, USA), digested with N-acetyl-L-cysteine/sodium citrate/sodium hydroxide for 20 minutes, concentrated by centrifugation at $5{,}000 \times G$ for 15 min, and examined according to recommended procedures (Kent and Kubica, 1985). Two smears were prepared from concentrated specimen sediments. One was heat-fixed, stained using fluorescent Auramine-O (AO) (Kent and Kubica, 1985), and examined microscopically

for acid-fast bacteria (AFB). When AO smears were positive or suspicious for AFB, the second smear was stained using the Ziehl-Neelsen method for confirmation. All specimen sediments were resuspended with 1.5 ml phosphate buffered saline (PBS), and equal aliquots were inoculated to a Lowenstein-Jensen medium slant (Becton-Dickinson, Cockeysville, Maryland, USA), a Middlebrook 7H11S medium slant (Becton-Dickinson), and a Bactec 12B broth vial (Becton-Dickinson, Sparks, Maryland, USA). All media were examined for growth, at least weekly, for 8 weeks. Bacterial growth was examined microscopically using a Ziehl-Neelsen stained smear to determine if growth was due to AFB. Acid-fast bacterial growth was subsequently tested by genetic probe (Accuprobe, Gen-Probe, San Diego, California, USA) to differentiate *M. tuberculosis* complex bacteria from other *Mycobacterium* spp. (Risner et al., 1994). Additional species identification was performed by biochemical testing and high-performance liquid chromatography (Butler et al., 1991) to distinguish *M. bovis* from other members of the *M. tuberculosis* complex and from other mycobacteria (Butler et al., 1991, Metchock et al., 1995).

Histopathology, performed only on heads that cultured positive for *M. bovis*, was carried out at the Animal Health Diagnostic Laboratory (AHDL), Michigan State University, as previously described (Fitzgerald et al., 2000). Formalin-fixed samples were paraffin-embedded, sectioned at 5 μm, and stained with hematoxylin and eosin as well as with Ziehl-Neelsen stain for microscopic evaluation. All samples were examined by a board-certified member of the American College of Veterinary Pathologists.

Determination of true prevalence in a population requires knowledge of apparent prevalence (as detected by some screening test) and of the sensitivity and specificity of that test (Rogan and Gladen, 1978). In this study, the existing surveillance protocol was considered the screening test, per O'Brien et al. (2004), and was evaluated against the gold standard test of mycobacterial culture. Sensitivity, specificity, and positive and negative predictive values were calculated as described by Thrusfield (1995), using freely available software (Lowry, 2007), with 95% percent confidence limits for all quantities calculated by the continuity-corrected efficient-score method (Newcombe, 1998). Sensitivity is the proportion of bovine TB culture-positive elk that is designated positive by screening, while specificity is the proportion of bovine TB culture-negative elk that test negative upon screening. Positive predictive value is the probability that an elk testing positive on screening actually is bovine TB-positive on culture, while negative predictive value is the probability that a test-negative screened deer actually is bovine TB-negative. For calculation of sensitivity, specificity, and predictive values, both nonsuspects (i.e. elk without gross lesions) and culture-negative suspects are considered bovine TB-negative by the existing surveillance protocol. Apparent prevalence (the number of grossly lesioned, *M. bovis* culture-positive specimens/total number of specimens tested), sensitivity, and specificity were used to calculate the true prevalence of bovine TB in the elk population per Rogan and Gladen (1978):

$$\hat{p} = \frac{\hat{t} + \beta - 1}{\alpha + \beta - 1}$$

where $\hat{p}$ = true prevalence, $\hat{t}$ = apparent prevalence, β = specificity and α = sensitivity.

RESULTS

In all, 334 elk from six counties (Charlevoix, Cheboygan, Missaukee, Montmorency, Otsego, and Presque Isle) were included in the study (Fig. 1). All but the Missaukee County elk were from the primary elk range. White-tailed deer infected with *M. bovis* have been documented in three of these counties (Montmorency, Otsego, and Presque Isle), and it is quite likely that infected deer are also present (albeit to a lesser extent), but as yet undetected, in the other three counties. One hundred eighty-nine (57%) of the study elk were female. Bulls ranged from 0.5 to 14.5 yr of age, with quartile breaks of 2.5, 4.5, and 5.5, while cows were from 0.5 to 17.5 yr, with quartiles of 2.5, 3.5, and 5.5. Twenty-three animals bore gross lesions and were considered bovine TB suspects. All were cranial and unilateral, with no predilection to side. Nineteen (83%) were in the palatine tonsils (10 right, nine left), three (13%) were in the medial retropharyngeal lymph nodes (one right, two left), and one (4%) was in the right submandibular lymph node. Lesions ranged in character from

TABLE 1. Cross-classification of the results of this study (rows) vs. the existing *Mycobacterium bovis* surveillance protocol (columns), of free-ranging elk, in Michigan, USA. For calculation of sensitivity, specificity, and predictive values, both nonsuspects and culture-negative suspects, are considered negative by the existing surveillance protocol, and are summed.

| This study | Existing surveillance protocol | | | |
| | Gross lesions present (suspects) | | Gross lesions absent (nonsuspects) | Total |
	Culture-positive	Culture-negative		
Positive	0	0	2	2
Negative	0	23	309	332
Total	0	23	311	334

diffuse mucopurulent areas with 1–2 mm white to yellowish concretions, to caseogranulomas up to 2 cm in diameter. All 23 elk with gross lesions were negative for *M. bovis* on mycobacterial culture.

Two elk, a 3-yr-old bull killed by a vehicle in June 2003 and a 2.5-year-old cow harvested by a hunter in December 2003, were culture-positive for *M. bovis*. Neither had gross lesions, nor were there any histopathologic lesions in the bull. Microscopic examination of the cow's tonsil was normal, but several sections, taken from multiple lymph nodes, contained scattered multifocal aggregates of foamy macrophages and multinucleate giant cells, present within both the cortex and medulla. Rarely, these giant cells showed AFB. No evidence of necrosis was found in any tissue section. Acid-fast bacteria were identified at MDCH-ML only from culture; tissue smears from both animals were negative.

A cross-tabulation, comparing culture results with the classification of elk by the current bovine TB surveillance protocol, is presented in Table 1. The sensitivity and specificity of the current surveillance protocol (95% confidence interval) were 0% (0, 80.2) and 100% (98.6, 100), respectively, while the predictive value of a negative test was 99.4% (97.6, 99.9). The failure of the surveillance protocol to detect either of the culture-positive elk resulted in division by zero in the positive predictive value calculation, and therefore in an undefined value. The period appar-ent prevalence of bovine TB, as detected by the current surveillance protocol, was 0%, while the true prevalence in the elk sampled was 0.6 % (0.1, 2.4) by direct calculation. Division by the 0% sensitivity in the formula of Rogan and Gladen (1978) resulted in an undefined period true prevalence of TB at the population level.

Several species of mycobacteria, other than *M. bovis*, were isolated from 47 of the 334 elk in this study. Of these, the most frequently occurring was *M. avium* complex (21/334 [6.3%]), followed by mycobacterial species other than tuberculosis (MOTTS; 15/334 [4.5%]), *M. terrae* complex (7/334 [2.1%]), *M. scrofulaceum* (2/334 [0.6%]), and *M. chelonae* ssp. *abscessus* (2/334 [0.6%]). Four elk with non-*bovis* mycobacterial isolates were among the 23 elk with gross lesions suggestive of TB. Two yielded *M. avium* complex, one a MOTT and the other *M. scrofulaceum*. The first three were tonsillar lesions, while the last had a lesion in the right medial retropharyngeal node. The remaining 43 elk, from which non-*bovis* mycobacteria were isolated, did not have gross lesions.

DISCUSSION

The limitations of this study, though few, should be kept in mind. Mycobacterial culture remains the gold standard for diagnosis of *M. bovis*. Polymerase chain reaction (PCR) techniques hold promise, but PCR has been shown to be markedly

less sensitive than culture (O'Brien et al., 2004). To the extent that bovine TB-positive elk in this study may have gone undetected by culture, the "true" prevalence noted here may be an underestimate. Because the majority of the elk in this study were hunter-harvested, and therefore unlikely to comprise an entirely random sample, these results may deviate somewhat from what might be found if it were possible to test the entire underlying population. This bias was mitigated to some extent, but not eliminated, by including non-harvest mortalities. Indeed, one of the two TB-positive elk was a road casualty.

Rogan and Gladen (1978) note that, in order to be classified as a test, a diagnostic process must select diseased individuals with higher probability than non-diseased individuals. Stated differently, as a group, individuals with positive tests are expected to have a higher disease prevalence than the sampled population overall. By these criteria, the current TB surveillance protocol did not perform sufficiently well in this study to even be classified as a test. This disappointing performance, however, is in all likelihood an artifact of application to a relatively small sample in order to detect a disease present at only a very low prevalence. Other studies have used similar protocols to sensitively detect bovine TB in elk (Whiting and Tessaro, 1994; Rohonczy et al., 1996), suggesting the 0% sensitivity noted in this study is anomalous. Of the five bovine TB-positive free-ranging Michigan elk detected thus far among 1,693 tested since surveillance began in 1996, three (a 5.5-year-old cow, a 3.5-year-old bull, and a 4.5-year-old bull, all harvested by hunters, in December 2000, September 2001, and December 2006, respectively), had gross lesions that were detected by the currently used protocol. The diagnostic findings in four of five bovine TB-positive Michigan elk suggest that they were in the early stages of infection, and so likely were excreting few TB bacilli (Lugton et al., 1998). The pathology of TB in both elk (e.g. Rhyan et al., 1992; Whiting and Tessaro, 1994; Rohonczy et al., 1996) and red deer (e.g. de Lisle et al., 1983; Clifton-Hadley and Wilesmith, 1991; Lugton et al., 1998) has been described in great detail elsewhere.

O'Brien and coworkers (2004) found seven of 754 WTD, without gross tuberculous lesions, to be positive for *M. bovis* on culture. That study documented the currently used surveillance protocol to be 75% sensitive, 100% specific, and to have positive and negative predictive values of 100% and 99.1%, respectively, in a population where the true prevalence of bovine TB was estimated to be 3.6% at the time of the study. Similar specificity and negative predictive values were observed in the present study. Had bovine TB prevalence exceeded 0.6%, or had more elk been available for testing (to increase the statistical power), sensitivity and positive predictive value would almost certainly have been higher. As with deer, the high predictive value of a negative test in the elk (99.4%) is important from a disease-management standpoint. The ability to assure a hunter, whose elk has tested TB-negative, that there is a <1% probability that the test result was erroneous is of inestimable importance in maintaining agency credibility. Moreover, with MDNR TB surveillance and control efforts under constant scrutiny by hunters, livestock producers, and state and federal agriculture agencies, it confers a high degree of validity to reported surveillance results.

An appreciable number of *Mycobacterium* spp. other than *M. bovis* were cultured from the elk tested in this study. Neither the number nor the variety is surprising, because many of these species can be commonly cultured from environmental specimens. The fact that 47 of 334 (14%) of the elk tested yielded mycobacterial species other than *M. bovis* suggests that differential speciation of AFB by microbiologic or molecular methods is essential to ensure accurate diagnosis of bovine TB in elk. The mere presence of

compatible gross lesions or of AFB is not reliable.

To date, WTD are the only free-ranging wildlife species shown to be a maintenance host for bovine TB in Michigan and a reservoir of infection for other species (O'Brien et al., 2006). Global experience with bovine TB in elk and red deer provides abundant evidence for their ability to act as maintenance hosts. However, the low prevalence of bovine TB in Michigan elk, and the early stage of pathogenesis in which the few infected animals have been found, does not suggest Michigan elk are anything more than spillover hosts at the present time. In assessing the overall risk of elk as a reservoir for bovine TB in Michigan, several factors support greater confidence in the conclusions drawn than is currently possible with WTD. The greater physical size and visibility of elk to the public make it much-less likely that significant causes of nonharvest mortality would go undetected or escape investigation by MDNR WDL. The mandatory carcass check and testing of all hunter-harvested elk, greater public and MDNR oversight of the elk hunt and hunters, and the inspection of elk gut piles for signs of bovine TB all make it much-less likely that harvest of an elk with gross lesions of bovine TB would go undetected. Finally, elk comprise a dramatically smaller population for sampling, enabling WDL to test a much-higher fraction of that population for TB. The size of the elk population during our study period can be estimated using a 2006 point estimate (Walsh, 2007), minus annual harvest and non-harvest mortality for 2002–2005, and adding an annual rate of population increase of 16% (MDNR, unpubl. data). These calculations yield reconstructed prehunt population estimates of 964, 970, and 1,019 for 2002–2004, respectively. The fractions of the population sampled annually in our study were 12.2, 9.8, and 10.7 over the same period. Taken together, all of these factors suggest it is unlikely that a major focus of bovine TB has gone undetected by current surveillance.

The principal bovine TB management goal for the elk herd is to minimize exposure by keeping elk densities as low as possible in the southeastern part of their primary range adjacent to DMU452. This has been accomplished, since 2001, by making more elk hunting licenses available in this area. The goal is also supported through minimization of WTD densities in the elk range, and through stiffer enforcement of baiting and feeding restrictions. Maintaining public support for these often-unpopular strategies is proving a formidable challenge (O'Brien et al., 2006), but will be crucial over the long term. Baiting and feeding of elk are prohibited in Michigan, but elk are known to help themselves opportunistically to bait or feed piles intended for deer. It is plausible, if not likely, that the elk infected with *M. bovis* thus far have ingested food items intended for, and contaminated by, bovine TB-infected deer. Deer and elk typically maintain spatial separation, and therefore have a low potential for direct transmission of bovine TB in the absence of some strong congregating factor (Miller, 2002). Whether elk remain spillover hosts in the future, or eventually become maintenance hosts may, in the end, have more to do with WTD management than management of the elk herd itself.

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