Prevalence of *Mycobacterium bovis* infection in cervids on privately owned ranches

John B. Kaneene, DVM, MPH, PhD; Michael VanderKlok, DVM; Colleen S. Bruning-Fann, DVM, MS, DACVP; Mitchell V. Palmer, DVM, PhD; Diana L. Whipple, MS; Stephen M. Schmitt, DVM, MS; RoseAnn Miller, BS

**Objective**—To determine prevalence of tuberculosis caused by infection with *Mycobacterium bovis* in cervids on privately owned ranches in northeastern lower Michigan.

**Design**—Epidemiologic survey.

**Animals**—Cervids on 96 privately owned ranches.

**Procedures**—A combination of slaughter and skin tuberculin testing was used to collect data. Infection with *M. bovis* was confirmed by use of standard necropsy and bacteriologic culture techniques.

**Results**—Cervids with tuberculosis were detected on 1 of the 96 ranches. The apparent prevalence of tuberculosis in cervids from the 96 ranches was 1.1 cases/100 cervids (21 cases/1,867 cervids tested). For the ranch with infected cervids, prevalence of infection with *M. bovis* was 12.1 cases/100 cervids (21 cases/174 cervids tested). No obvious gross lesions were seen in 8 of 21 white-tailed deer and 1 coyote with culture-confirmed *M. bovis* infection.

**Conclusions and Clinical Relevance**—The lack of visible lesions in a substantial proportion of infected animals should be taken into consideration in studies involving detection and prevalence of tuberculosis. (Am J Vet Med Assoc 2002;220:656–659)

Farming of privately owned cervids (deer and elk) is an industry that has been expanding in the state of Michigan. Because of the increasing importance of this industry, cervid farming was recognized by the state as an agricultural endeavor in 2000, which provided cervid farmers with the benefits of agricultural recognition and support from the Michigan Department of Agriculture. This recognition also made it easier for cervid farmers with the benefits of agricultural recognition and support from the Michigan Department of Agriculture.

In 1975, tuberculosis (TB) caused by *Mycobacterium bovis* was diagnosed in a wild white-tailed deer (*Odocoileus virginianus*) killed by a hunter in northeastern lower Michigan. This case was thought to result from contact with infected cattle, because the disease had only recently been eradicated from Michigan, and no follow-up investigation was performed. In the fall of 1994, the second recognized case of TB in a hunter-killed wild white-tailed deer in Michigan was found.

Since 1994, cooperative studies of TB in Michigan deer have been conducted by Michigan State University, the Michigan Department of Natural Resources, the Michigan Department of Community Health, the Michigan Department of Agriculture, and the USDA: Animal and Plant Health Inspection Service (APHIS), Veterinary Services. These studies revealed endemic TB in the wild white-tailed deer population of northeastern Michigan, and surveillance of livestock in the TB-affected area began in 1995. It is believed that the infection rate has been low in this deer population for decades, and infection was only recognized when prevalence became high enough to be detected through normal deer harvesting. Recognition of this wildlife reservoir and the potential for spread of this disease to livestock necessitated a TB surveillance program for livestock, privately owned cervids, and other wildlife species in the area.

Farms in the TB-affected area are likely to be surrounded by a free-ranging reservoir of TB, which can result in contact between privately owned stock and infected wild deer. Some large-acreage cervid ranches in the area that were not able to drive free-ranging deer from their property fenced the property, purchased local wild white-tailed deer from the state, and thus may have inadvertently introduced *M. bovis*-infected animals into their herds. The rapid increase in numbers of cervid farms in the 1990s also coincided with the presumed increase in TB in the wild deer population, increasing the likelihood that wild deer could have introduced TB into a privately owned cervid ranch.

The objective of this study was to use data from routine TB surveillance programs to determine prevalence of TB caused by *M. bovis* in cervids on privately owned ranches in northeastern lower Michigan. We hypothesized that such information could form a base of knowledge that can be used to improve ranch biosafety and management practices.
Materials and Methods

The initial goal of livestock surveillance in the TB-affected area was testing of all cattle, goats, and privately owned cervids, which was begun in 1995. This area presently includes a 5-county area where TB is endemic in wild white-tailed deer and a buffer zone around the endemic counties; this region is collectively referred to as the high-risk area (Fig 1). When livestock surveillance revealed infected herds, movement tracing and epidemiologic investigations were used to identify potential TB-source or TB-exposed herds, which were also tested. All privately owned cervid ranches in the TB-endemic 5-county area were tested first, followed by all ranches within the buffer zone.

Surveillance methods—Whole-herd single cervical tuberculin (SCT) tests, comparative cervical tuberculin (CCT) tests, or slaughter surveillance was used. Each ranch owner, with assistance from the Michigan Department of Natural Resources, was allowed to select between skin testing and slaughter for the cervids on their facilities. Classification of ranches as TB-positive or TB-negative via SCT testing was conducted according to procedures outlined by the USDA. The slaughter surveillance protocols were modifications of the USDA slaughter surveillance described for captive cervid facilities under a Modified Herd Plan. For ranches to be qualified as TB-free, negative results of a SCT test for all cervids sampled were the standard. However, when slaughter surveillance was used, the slaughter test was considered a positive test if 1 or more infected animals were detected.

Single cervical tuberculin test surveillance—This surveillance method was used at ranches with facilities for handling live cervids and applied to all animals 12 months of age and older. Cervids tested by use of the SCT test were classified as having negative or suspect results. If a suspect result was found, the animal could be either retested with a CCT test or necropsied. Reactors to the CCT test would also be necropsied. On the basis of a report by the Scientific Advisory Subcommittee of the Committee on Tuberculosis of the United States Animal Health Association, the SCT test in combination with CCT test has a test sensitivity from 75 to 85% for cervids.

Slaughter surveillance—This surveillance method was used at ranches that could not feasibly conduct whole-herd skin testing and was conducted by examining tissues from the number of culled animals equal to that necessary to detect TB in the herd at a prevalence of 2% with 95% confidence during a 3-year period, which is the number required by the USDA to establish an official TB-monitored herd. Owners submitted heads, and often thoracic viscera, to Michigan Department of Agriculture and USDA personnel, who retrieved the retropharyngeal, mandibular, parotid, and, if possible, tracheobronchial lymph nodes, and any tissues with lesions. Necropsy tissues were examined histologically (including acid-fast staining) and submitted for bacteriologic culture, mycobacterial identification, and molecular typing at the USDA, National Veterinary Services Laboratory, in Ames, Iowa. Sensitivity and specificity of this method in cervids are not known, but for comparison, sensitivity of examining only the cervical lymph nodes of deer is 43%, sensitivity of routine meat inspection ranges from 53 to 67%, sensitivity of complete necropsy in cattle is 93%, and collection of the parotid, mandibular, retropharyngeal, and tracheobronchial lymph nodes with histologic examination and mycobacterial culture would have detected 10 of 15 cattle naturally infected with M bovis.

Determination of TB status and prevalence—A TB-infected animal was defined as 1 in which M bovis infection was confirmed by use of mycobacterial culture techniques. A TB-infected ranch was defined as 1 that had 1 or more animals in which M bovis infection was confirmed by use of mycobacterial culture techniques. Prevalence of TB was computed as the number of positive results divided by the number of tests times 100.

Results

Ninety-six privately owned cervid ranches in the high-risk area were tested from 1995 to 2000. Most cervids tested were white-tailed deer, with elk and other species making up approximately 20 and 2% of all cervids tested, respectively. During this time, 328 of 35,517 white-tailed deer from the high-risk area were found to be infected with M bovis. Mean herd size of ranches under SCT test surveillance was 26.4 cervids, whereas mean herd size of ranches under slaughter surveillance was 146.7 cervids (Table 1). In the buffer zone, there were 34 SCT test suspects among 663 animals under skin test surveillance and no positive results among 247 animals under slaughter surveillance. One of the 34 SCT test suspect animals yielded positive results when retested by use of the CCT test. This animal was subsequently necropsied, and M bovis was not detected. Tuberculosis was not confirmed in any animals in the buffer zone.

In the endemic area, there were 37 SCT test suspects and no CCT test reactors among the 575 animals under skin test surveillance. Tuberculosis was detected on 1 privately owned cervid ranch located in the endemic area in 1997 when M bovis infection was confirmed by use of mycobacterial culture techniques in tissue samples from a white-tailed deer carcass with gross lesions that were submitted by the ranch owner. All detection of TB on this ranch was slaughter-based, because the size of the ranch (6.04 km²) and lack of handling facilities precluded skin testing. Of 325 cervids on the ranch, mostly white-tailed deer with 6 elk, 3 sika deer, and 1 fallow deer, 174 were necropsied and examined for TB. Details on a subset of 116 of the 174 cervids have been reported. Twenty-one of 174 (12.1%) cervids had positive results of mycobacterial culture; 8 of these cervids did not have gross lesions of TB. Comparison of isolates from deer on the property with isolates from wild white-tailed deer in the high-

![Figure 1—Map of Michigan (inset) and counties in northeastern lower Michigan. Notice the 5-county area where tuberculosis in white-tailed deer is endemic and the surrounding buffer zone, collectively referred to as the high-risk area.](Image 75x113 to 289x267)
risk area by use of restriction fragment length polymorphism analysis revealed that the isolates were identical. In addition to M bovis, samples from 1 infected deer also yielded M avium, and samples from 1 deer with negative results for M bovis yielded M terrae. During the herd depopulation process, 2 coyotes were shot on the TB-positive ranch and necropsied. Gross or histologic lesions were not seen in any of these animals, but M bovis was identified via bacteriologic culture of pooled lymph nodes from 1 coyote.

On Sep 1, 1998, environmental samples were collected from this site for isolation of M bovis. Sixty-one samples, including soil, feed, fecal material, and water were collected for mycobacterial culture; none of the samples, including soil, feed, fecal material, and water yielded M bovis. Environmental samples were also taken during slaughter surveillance for isolation of M bovis in December 1998, and none of these samples yielded M bovis in culture.

Ranch prevalence of TB in the high-risk area was 1/96 from 1995 to 2000, determined on the basis of at least 1 test for each herd. The apparent prevalence of TB in individual deer from all ranches in the high-risk area was 1.1 cases/100 cervids (21 cases/1,867 cervids tested). On the TB-positive ranch, prevalence of M bovis infection was 12.1 cases/100 cervids (21 cases/174 cervids tested).

### Discussion

Prevalence of TB in privately owned cervid ranches in the high-risk area and apparent prevalence in cervids were low (1.0 and 1.1%, respectively). Since 1997, only 1 ranch with infected animals has been found, which raises the question of why this particular herd was infected. The initial herd comprised 108 wild white-tailed deer fenced in by the ranch in 1992. In 1995, TB was determined to be endemic in an area approximately 12 km south of this property. It is likely that 1 or more of the original 108 deer were already infected with M bovis, which went undetected until the herd prevalence increased and was detectable through the use of trained personnel and proper facilities for necropsy, histologic examination, and mycobacterial culture.

The major advantages of SCT testing are that it ensures that most of the herd population is tested and does not result in automatic loss of tested animals. This may be preferred by a ranch owner whose normal culling rates do not provide the sample size needed for slaughter surveillance and who may resist losing animals to slaughter surveillance. However, the ranch must have deer handling capabilities so that the test can be administered and read 72 hours later. All adult animals must be tested, which requires extra labor and time and places great stress on the animals. If retesting is required because of suspect results, the second test must be administered within 10 days or after 90 days from the first test, leaving an 80-day period during which testing cannot proceed. Also, the sensitivity of the SCT test with the CCT test is 65 to 75% (in cervids), compared with 93% for total necropsy (in cattle), thereby increasing the likelihood that TB in an individual animal may be missed.

A possible alternative method of SCT testing for cervids may be to initially test animals with the CCT test. Although the 72-hour waiting period between test administration and evaluation must be maintained, results of recent research indicate that the CCT used in experimentally infected white-tailed deer has a sensitivity and specificity of 97% and 91%, respectively. This method would remove the need for retesting of suspect animals, and the reported test sensitivity is an improvement over the 65 to 75% sensitivity of the SCT in combination with the CCT.

In comparison with SCT testing, slaughter surveillance may be the only viable method for ranches that do not have cervid handling capabilities. Slaughter surveillance can minimize inconvenience to a ranch, particularly if the number of deer targeted for routine culling is adequate for surveillance. Most naturally reproducing mature white-tailed deer herds with 100 or more adult animals can yield the required sample numbers through routine management and harvesting.

The main disadvantages of slaughter surveillance are the number and selection of animals for surveillance. If the numbers required for adequate surveillance exceed normal culling rates on the ranch, extra animals will have to be removed to meet the minimum testing requirements outlined by the USDA. Use of only owner-submitted cull animals may provide a nonrepresentative sample of the deer population if selection is based on age or sex (ie, leaving older males) or if selection of only smaller or unthrifty animals is practiced. Another disadvantage is the expense incurred through the use of trained personnel and proper facilities for necropsy, histologic examination, and mycobacterial culture.

Considering the different strengths and weaknesses inherent in skin test and slaughter surveillance, the question arises as to which surveillance method is the most efficient. Unfortunately, the direct comparison necessary to answer this question was not possible, because none of the ranches used both surveillance methods simultaneously. This policy of using different surveillance methods differs from that in other coun-

---

**Table 1—Results of tuberculosis testing in cervids in a high-risk area of Michigan, 1995–2000**

<table>
<thead>
<tr>
<th>Testing area</th>
<th>Skin test surveillance</th>
<th>Slaughter surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ranchoes (No.)</td>
<td>Cervids (No.)</td>
</tr>
<tr>
<td>Endemic</td>
<td>25</td>
<td>704</td>
</tr>
<tr>
<td>Buffer zone</td>
<td>40</td>
<td>1,014</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>1,718</td>
</tr>
</tbody>
</table>

*Percentage of animals classified as suspect on the basis of results of single cervical tuberculin tests.
†Percentage of animals classified as reactors on the basis of results of comparative cervical tuberculin tests.
‡Percentage of animals classified as tuberculosis-positive on the basis of results of mycobacterial culture.
tries, such as New Zealand, where surveillance is standardized (skin and TB blood testing) for the entire captive cervid population.

Regardless of the method of testing used in surveillance, the accepted gold standard for determination of TB status is bacteriologic culture of \textit{M bovis}. Although culture confirmation has been used for years, concerns have been raised regarding its use as the primary method to verify TB. Time required for culture of \textit{M bovis} is long (6 to 8 weeks) because of slow growth of the organism in media. This causes delays in the implementation of control measures and can cause hardships for ranches that may remain under quarantine until confirmation of TB status. Facilities capable of conducting mycobacterial culture testing are limited to a few certified laboratories with the necessary equipment, supplies, and trained personnel. The shipping time between collection of specimens and the actual processing of specimens may become a problem, particularly for specimens taken in the field prior to shipment. Additionally, testing of samples may be delayed when other projects with higher priority occupy laboratory time and incubator space. This problem has been seen with wild white-tailed deer surveillance, particularly during firearm deer hunting season, when the quantities of specimens being tested can overwhelm testing facility capacity. A possible solution to the time constraints seen in mycobacterial culture testing is the use of rapid, highly sensitive genetic tools, such as gene probes and spoligotyping, for the identification of \textit{M bovis}.

One potential area of concern is the absence of gross lesions in animals infected with \textit{M bovis}. Since no visible lesions were present in 8 of 21 infected deer in our study, it is likely that \textit{M bovis} may be missed if samples submitted for culture are limited to animals with gross lesions. However, if lymph nodes from all necropsied animals are subjected to mycobacterial culture, the likelihood of detecting infection is quite good and should detect \textit{M bovis} despite the lack of visible lesions.

The \textit{M bovis} surveillance program for privately owned cervids in the state of Michigan appears to be effective. The finding of a TB-positive privately owned cervid ranch through slaughter surveillance indicates the effectiveness of this approach. There are areas in which various aspects of the surveillance process can be improved, but these should not limit the program's ability to identify ranches with infected animals. With constructive changes in surveillance methods and experience gained during the surveillance process, the efficiency of the privately owned cervid surveillance program in Michigan should continue to improve.

References