The Michigan Bovine Tuberculosis Problem
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Abstract: Since the initial discovery of a wild white-tailed deer (Odocoileus virginianus) infected with Mycobacterium bovis during 1994 in Michigan, USA, we have conducted annual tuberculosis surveillance in wildlife. This surveillance has primarily utilized hunter-killed deer and elk submissions. While surveillance is conducted statewide, the most intense surveillance has focused on the endemic 5-county area. Our survey is conducted by a multi-institutional task force, composed of personnel from: the MI Department of Natural Resources, the MI Department of Agriculture, the MI Department of Community Health, the United States Dept. of Agriculture, and the Diagnostic Center for Population and Animal Health, Michigan State University. This joint task force allows for integrated policy and management decisions for tuberculosis surveillance, research and eradication efforts.

After nine years of surveillance and research, we have established a number of important principals and associations regarding bovine tuberculosis in wild deer. 1) Wild white-tailed deer can serve as maintenance and reservoir hosts for M. bovis. 2) Supplemental feeding of deer tends to increase disease prevalence within the wild population. 3) Ingestion of M. bovis contaminated feed may be more important than droplet inhalation transmission both between deer, and between deer and cattle. 4) The spatial distribution of infected deer tends to be irregular or clumped into “hot-spots” rather than uniformly distributed. 5) Once tuberculosis has become established in a wild deer population, management practices such as stopping supplemental feeding, and decreasing population density will reduce the prevalence of the disease, but may not completely eradicate the disease.

Surveillance:
Michigan (MI) had been a United States Department of Agriculture tuberculosis accredited-free state since 1979, meaning no tuberculous cattle had been detected during the previous five years. During the fall of 1994 a hunter harvested a wild white-tailed deer, and upon opening the carcass found disseminated granulomas throughout the lungs and pleura (Schmitt et al 1997). We diagnosed granulomatous pneumonia with occasional acid-fast bacilli and so began our involvement with Mycobacterium bovis in wild white-tailed deer.

Since bovine tuberculosis in North America had previously been considered a domestic cattle disease with only occasional spill-over into wild deer, it was not considered likely that the disease would maintain itself in the wild deer population. After consultation with epidemiologists and statisticians, it was determined that a survey of a least 300 wild deer within a 16 km radius of the index case would indicate whether this was an isolated case or part of an outbreak. During the fall hunt of 1995, 386 deer were submitted from the area for tuberculosis surveillance (Schmitt et al 1997). This initial surveillance depended on hunter-submitted heads, from which lymph nodes were collected for routine H&E microscopic examination, acid-fast stains, and mycobacterial isolation. Unfortunately, 18 culture-positive deer were detected, and we realized that M. bovis was endemic in wild white-tailed deer in this locale. A deer management area (DMU452) was designed to encompass the entire known infected population, and we began to revise and refine our annual surveillance quotas, surveillance techniques, as well as develop management strategies to control and eventually eliminate this zoonotic disease.

Our surveillance team is composed of personnel from a number of disciplines and agencies. Personnel with the MI Department of Natural Resources (DNR) handle field collection, identification, and hunter notification for the wild deer, elk and carnivore surveys. Veterinarians and technicians with the MI Department of Agriculture (MDA) as well as the United States Dept. of Agriculture (USDA) have primary responsibility for skin testing and indemnity of cattle and captive cervids. Personnel with the MI
Department of Community Health have performed the mycobacterial isolation and identification from wildlife samples, while scientists with USDA at Ames, Iowa, handle samples from domestic animals. Epidemiologists from the Population Medicine Center, Michigan State University, and the local USDA office participated in the design and analysis of the survey data. Finally, personnel with the Diagnostic Center for Population and Animal Health (DCPAH), Michigan State University, perform necropsy examination and microscopic examination of suspect tissues for both domestic and wild animals.

Tuberculosis surveillance is based on hunter-harvested deer and elk. Target quotas for each geographic region of the state were selected at the 95% confidence level in order to detect tuberculosis prevalence rates of 0.2% or higher. Successful hunters stop at roadside check stations, where carcasses are quickly examined by DNR personnel to detect disseminated cases of tuberculosis. Also at these stations deer heads are solicited from hunters willing to participate in the voluntary program, marked with individual identification tags, and information obtained on the one square mile where the animal was harvested as well as hunter contact information. Heads are initially processed to identify the animal’s sex and approximate age based on its teeth, then are forwarded to the DCPAH. Once at the DCPAH, cranial lymph nodes are examined for abscesses or granulomas. Lesioned lymph nodes are collected for routine H&E staining, acid-fast staining, and mycobacterial isolation and identification.

The apparent prevalence in the core area of endemic infection has seemingly plateaued since 1998 in the 2.3-2.8% range (Table 1). During the 2000 and 2001 hunting seasons we ran a study to estimate the true prevalence of tuberculosis. We selected six townships from the core area that exhibited high apparent prevalence rates of tuberculosis over the first 5 years of the study; these six townships had apparent prevalence rates ranging from 3.6 to 7.1% (O’Brien et al In press). All deer with no gross lesions examined during the fall surveillance programs in 2000 and 2001 which were harvested in those six townships had mycobacterial isolation performed, and subsequently, all culture positive animals were also examined histologically. Of the 701 deer included in this study, an additional seven positive deer were detected which would have been missed based on the gross screening technique routinely used. The apparent prevalence of tuberculosis in those two townships was 2.5%, however, the added diagnostic testing pushed the true prevalence to 3.6%. Based on this study, we have increased our apparent prevalence by a 33% correction factor to calculate estimated true prevalence rates (Table1).

Table 1. Apparent prevalence and estimated true prevalence rates for infection with tuberculosis in Michigan’s wild white-tailed deer.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Deer</th>
<th>Total Pos. Deer</th>
<th>Core AP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Core TP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>5-Co AP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>5-Co TP&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>386</td>
<td>18</td>
<td>5.1%</td>
<td>6.8%</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1996</td>
<td>3,690</td>
<td>47</td>
<td>2.5%</td>
<td>3.3%</td>
<td>0.17%</td>
<td>0.23%</td>
</tr>
<tr>
<td>1997</td>
<td>3,518</td>
<td>67</td>
<td>4.4%</td>
<td>5.9%</td>
<td>0.43%</td>
<td>0.57%</td>
</tr>
<tr>
<td>1998</td>
<td>7,915</td>
<td>75</td>
<td>2.7%</td>
<td>3.6%</td>
<td>0.28%</td>
<td>0.37%</td>
</tr>
<tr>
<td>1999</td>
<td>17,502</td>
<td>56</td>
<td>2.3%</td>
<td>3.1%</td>
<td>0.19%</td>
<td>0.25%</td>
</tr>
<tr>
<td>2000</td>
<td>22,011</td>
<td>53</td>
<td>2.6%</td>
<td>3.5%</td>
<td>0.35%</td>
<td>0.47%</td>
</tr>
<tr>
<td>2001</td>
<td>24,278</td>
<td>60</td>
<td>2.3%</td>
<td>3.1%</td>
<td>0.55%</td>
<td>0.73%</td>
</tr>
<tr>
<td>2002</td>
<td>16,137</td>
<td>51</td>
<td>2.8%</td>
<td>3.7%</td>
<td>0.48%</td>
<td>0.64%</td>
</tr>
<tr>
<td>2003</td>
<td>16,000+</td>
<td>25+</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup> Apparent prevalence  
<sup>b</sup> True prevalence  
<sup>c</sup> data not available
Epidemiology and Management:
The fact that townships are highly variable in disease prevalence was not unexpected. The tuberculosis distribution in New Zealand’s brushtail possum population is similar. Several epidemiologic studies have been conducted to try and establish some of the factors responsible for non-uniformity or “hot spots”. We have long believed that congregating deer into high densities around supplemental feeding sites has encouraged the lateral transmission of tuberculosis. Two studies found association between increased feeding sites and increasing risk for bovine tuberculosis in deer (Hickling 2002; Miller et al 2003). Another more recent study established “hot spots” of bovine tuberculosis, however the association with supplemental feeding was not pronounced, although association with natural surface water was strong (Miller et al submitted). Other epidemiologic trends were that older bucks tend to have higher infection rates than both younger deer and does (O’Brien et al 2002).

The purpose of our surveillance work has been to evaluate our progress in reducing the prevalence of tuberculosis in wild deer, with the ultimate goal of eradicating the disease. A series of management initiatives have been established by the DNR in conjunction with the MDA and the state legislature. Initially, the formation of a new DMU to encompass the core endemic area was established in 1995. Then a series of initiatives were undertaken to reduce the deer population density and decrease lateral transmission of tuberculosis. The deer population reduction included additional rifle hunting seasons in October and December, increases in and discounted prices for antlerless licenses to reduce the doe population and hence the reproductive capacity, as well as increased Disease Control permits to harvest deer year round. Over a seven year period (1995-2001), the total estimated white-tailed deer population in the five central counties dropped from 165,000 to 100,000. To reduce lateral disease transmission, both baiting (placing food materials during hunting season as a deer lure), and supplemental feeding of deer (placing food materials to attract deer for any reason at any time of the year) were made illegal within a 7-county area containing 99% of all tuberculous deer. Aerial observations are conducted annually in Feb./March; these observations have confirmed significant reduction (approx. 75-80%) in number of large feeding sites, and less food was available at those sites still active.

Pathology and Diagnostics:
We have now examined over 120,000 wild white-tailed deer for tuberculosis, with over 450 positive individual animals. From this extensive surveillance, we have made several generalizations. First, the principal cranial lymph node involved is the medial retropharyngeal lymph node (approx.. 95% of time in tuberculous deer with cranial lesions), which may be affected unilaterally or bilaterally in approximately equal numbers (O’Brien et al 2001). Seventy-five percent of all tuberculous animals had cranial lymph node lesions. Forty-five percent of infected animals had some extracranial lesions of tuberculosis, with the lung and pleura being the most frequently affected extracranial tissues. While gross lesions typically exhibit purulent or liquid centers and resemble classic abscesses, histologic examination reveals that virtually all lesions are actually caseogranulomas (Fitzgerald et al 2000). The central necrotic material is commonly partially mineralized; frequent enough to be a useful indicator for tuberculosis. The inflammatory cells surrounding the necrotic core are predominantly histiocytes, lymphocytes, and multinucleated giant cells; again, the presence of giant cells is associated frequently enough to be a useful indicator. On the other hand, primarily suppurative lesions and the presence of Splendore-Hoeppli material are virtually never associated with tuberculosis. Acid-fast staining (Ziehl-Neelsen) is useful to demonstrate Mycobacterium sp. organisms, which are generally rare to uncommon.

Our full diagnostic procedure involves gross examination of cranial lymph nodes, and additional tissues if provided, and gathering both formalin-fixed and fresh samples from lesions. Formalin-fixed tissues are stained by routine H&E as well as acid-fast stains, and examined by a board-certified pathologist. Fresh samples proceed to the mycobacterial isolation laboratory, and an automated Bactec vial system (Becton-Dickinson, Sparks, Maryland, USA) in which media are checked for growth on a weekly basis for up to 8 weeks; most positive cultures were detected within 2-4 weeks incubation. Subsequently a M.
**tuberculosis** group-specific DNA probe (Accuprobies, Gen-Probe, San Diego, California, USA) was used to confirm the presence of *M. tuberculosis* group organisms. Final species identification utilized a series of biochemical assays and high performance liquid chromatography. While isolation and identification has long been considered the gold standard for *M. bovis* testing, we have found histopathology to be rapid and sensitive (98%), and that the genetic probe has proven to be 100% sensitive and specific in our wild deer surveillance program (Fitzgerald et al 2000).

**Other Species:**

While wild white-tailed deer have proven to be the principal reservoir host in Michigan, the problem with tuberculosis is not limited to this wild population alone. There are ongoing surveillance programs in wild elk, captive cervids, wild carnivores, and domestic cattle. The elk population in Michigan is relatively small (estimated winter herd of 900 animals) and restricted to a limited geographic region, which unfortunately overlaps with the endemically infected deer population. Approximately 150 elk are harvested by permit each fall, all of which are surveyed for tuberculosis using methods very similar to the deer survey. Of the over 1,200 elk surveyed since 1996, only three positive individuals have been detected. Two of these appeared to be an early stage infection in which only the tonsil was infected, while a second animal had medial retropharyngeal lymph node involvement. More serious has been the secondary spill-over of *M. bovis* infection into wild carnivores and omnivores (Bruning-Fann et al 2001). Nearly 1,500 wild carnivores and omnivores have been surveyed from the 5-Co. area, with 42 positive animals including 18 coyotes, 8 raccoons, 7 black bear, 4 bobcat, 2 opossum and 3 red fox. Most of these animals had neither gross nor histologic lesions; in those few animals with histologic lesions, the lesions were limited to the mesenteric lymph nodes. These findings suggest that the principal route of infection is through ingestion of contaminated carcasses or offal. We believe that none of the carnivore species identified to date are multiplier hosts, and that few if any of these individuals are even shedding the bacteria.

We recognize that carnivore/omnivore hosts play a critical role in the transmission and maintenance of bovine tuberculosis in a number of countries. Therefore, in addition to our surveillance of a variety of wildlife species in Michigan, we have conducted a series of experimental inoculation studies under BL-3 conditions in common wildlife species. This includes wild rodents (Norway rats, house mice, and meadow voles), Virginia opossums, raccoons, as well as common bird species (European starlings, common crows, pigeons/rock doves, mallard ducks, and wild turkeys). Certainly, rodents are ubiquitous and many laboratory animal strains are known to be highly susceptible to *M. bovis* infection. We found voles to be highly susceptible, mice moderately susceptible, and rats to be nearly resistant. The opossum, distant relative of the brushtail possum that plays a major role in New Zealand, to be moderately susceptible although not affected by high mortality and widespread shedding of the organism (Diegel et al 2002; Fitzgerald et al 2003). Most surprisingly, several bird species were susceptible to *M. bovis*. While birds are not nearly as susceptible to *M. bovis* as mammals are, our studies would rate their susceptibility to infections as follows: pigeons > crows and starlings > turkeys and mallards (Butler et al 2001; Fitzgerald et al 2003). Pigeons appear to pose the greatest threat as they shed *M. bovis* in their feces for at least 60 days post inoculation, and good certainly spread the disease over many miles in short periods of time. Furthermore, birds pose a significant bio-security risk due to their small size, high population numbers, and abilities to pass over or through many barriers in agriculture settings.

Since wild deer are highly susceptible to bovine tuberculosis, it comes as no surprise that captive deer are similarly affected. There are 70 privately owned deer herds in the 5-Co. endemic tuberculosis area of Michigan (Kaneene et al 2001). One of two surveillance plans are in place for these herds. Either they may undergo annual single cervical tuberculosis skin testing, or they have slaughter surveillance of all deer harvested or found dead. Since 1999, this mandatory captive cervid testing has been extended statewide; any herds not participating are placed under quarantine so no live animal movement may occur. Only one captive cervid herd has ever been found positive since 1994. That herd was in Presque Isle Co., an endemic county that historically has had tuberculosis prevalence rates.
between 0.5 and 1.0%. This herd was formed by enclosing approx. 1,500 acres in fence, and purchasing an estimated 118 wild white-tailed deer from the DNR that were entrapped behind the fence. We would extrapolate that only one or two infected deer were likely acquired from the wild. Seven years later, when this herd was depopulated due to tuberculosis, a tuberculosis prevalence of 12.0% was detected at necropsy (Palmer et al 2000). This single captive herd exhibits the potential role of supplemental feeding and high deer densities in spreading tuberculosis, because in only seven years the prevalence rose from around 0.5 - 1% to 12%.

Important Principals:
1) Wild white-tailed deer can and do serve as reservoir and maintenance hosts for *M. bovis*. While this may seem obvious to most everyone at this conference today, ten years ago in North America we were assured by all the wildlife experts that our index case was an escaped captive deer or a single isolated spill-over from infected domestic animals. It was very much news when we found endemic bovine tuberculosis in Michigan wild deer. Nor has long term and intensive wildlife surveillance found another wildlife reservoir host present in Michigan similar to those identified in other countries.

2) Supplemental feeding, causing abnormally high deer densities and close contact for prolonged periods seems to be an important factor in the tuberculosis endemic. While this association appears obvious, it is harder to scientifically prove. Several epidemiology studies confirm this association. The main difference between the endemic region of Michigan and other statewide areas is the intensive supplemental feeding. Secondly, we have the single captive herd which so rapidly increased its tuberculosis prevalence under similar feeding practices.

3) We believe that ingestion of contaminated feed may be of equal or greater importance than classic airborne transmission between deer, and from deer to cattle. This is based on the predominance of cranial lymph node involvement, experimental inoculation studies in deer, and the numerous deer to cattle transmissions of the disease without close spatial or temporal contact.

4) Uneven spatial distribution of the disease is a striking feature in Michigan, much as it is in New Zealand. This suggests that successful control of the disease will require control methods at a much finer geographic level than is presently done.

5) Finally, our experience with intensive and expensive management and surveillance of bovine tuberculosis in wild deer over the last ten years suggests that while we are controlling the disease, and even reducing the prevalence, complete eradication may not be possible. Innovative new strategies using focal deer depopulation, ante-mortem deer testing, or even oral bait vaccines may be necessary if eradication is the ultimate goal.

References:


