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Naturally occurring tuberculosis in white-tailed deer

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Objective—To determine the distribution of lesions and extent of tissues infected with *Mycobacterium bovis* in a captive population of white-tailed deer.

Design—Cross-sectional study.

Animals—116 captive white-tailed deer.

Procedure—Deer were euthanatized, and postmortem examinations were performed. Tissues with gross lesions suggestive of tuberculosis were collected for microscopic analysis and bacteriologic culture. Tissues from the head, thorax, and abdomen of deer with no gross lesions were pooled for bacteriologic culture. Tonsillar, nasal, oral, and rectal swab specimens, fecal samples, and samples of hay and pelleted feed, soil around feeding sites, and water from 2 natural ponds were collected for bacteriologic culture.

Results—*Mycobacterium bovis* was isolated from 14 of 116 (12%) deer; however, only 9 of 14 had lesions consistent with tuberculosis. Most commonly affected tissues included the medial retropharyngeal lymph node and lung. Five of 14 tuberculous deer had no gross lesions; however, *M bovis* was isolated from pooled tissue specimens from the heads of each of these deer. Bacteriologic culture of tonsillar swab specimens from 2 of the infected deer yielded *M bovis*. Mean (± SEM) age of tuberculous deer was 2.5 ± 0.3 years (range, 0.5 to 6 years). *Mycobacterium bovis* was not isolated from feed, soil, water, or fecal samples.

Conclusions and Clinical Relevance—Examination of hunter-killed white-tailed deer for tuberculosis commonly includes only the lymph nodes of the head. Results of such examinations may underestimate disease prevalence by as much as 57%. Such discrepancy should be considered when estimating disease prevalence. (*J Am Vet Med Assoc* 2000;216:1921–1924)

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Mycobacterium bovis is the causative agent of tuberculosis in cattle and has a broad host range that includes humans. In 1994, a wild white-tailed deer (*Odocoileus virginianus*) killed by a hunter in Alpena County, Mich, was found to have gross and microscopic lesions consistent with tuberculosis. *Mycobacterium bovis* was later isolated from affected tissues.[1] This prompted a survey in 1995 that resulted in the isolation of *M bovis* from 18 of 354 (5.1%) deer in Michigan. As of August 1999, more than 17,755 deer killed by hunters from the affected area of Michigan have been examined; 228 (1.3%) were found to be infected with *M bovis*. Prior to 1994, *M bovis* infection had been diagnosed sporadically in wild white-tailed or mule deer (*Odocoileus hemionus*) in North America.[2-5] In each of these reports, only 1 to 3 deer were affected. The Michigan outbreak represents the first known epidemic of *M bovis* in free-ranging white-tailed deer in North America. With more than 19 million white-tailed deer, 5 million mule deer, and 750,000 elk (*Cervus elaphus*) in the United States,[6] a reservoir of *M bovis* infection in free-ranging wildlife not only represents an emerging disease but also a threat to domestic livestock and the bovine tuberculosis eradication effort in the United States.

In 1997, tuberculosis was found in a captive herd of white-tailed deer in Presque Isle County, Mich, where tuberculosis is known to be endemic in free-ranging white-tailed deer. The herd had been formed in 1992 by fencing approximately 1,500 acres, including wild white-tailed deer residing in the area. Since then, the 1,500-acre facility had been operated as a private hunting establishment. Comparison of restriction fragment length polymorphism results of the *M bovis* isolates from these captive deer and wild deer in the surrounding area suggested that some of the wild resident deer confined within the original 1,500 acres had been infected with *M bovis*.[a] A plan was devised and implemented by the Michigan Department of Agriculture, the herd owner, and the USDA to humanely remove all deer from the premises.

The purpose of the study reported here was to describe tuberculosis in this population of naturally infected white-tailed deer through complete postmortem examination, including microscopic analysis

and bacteriologic culture of tissue specimens. A thorough understanding of the pathogenesis and transmission of *M bovis* among white-tailed deer will facilitate management decisions concerning this emerging disease problem.

Materials and Methods

Animals—One hundred sixteen deer were examined from a private herd of approximately 300 deer on a 1,500-acre fenced premises in northeast Michigan. The facility was operated as a commercial hunting retreat, and equipment for capture or restraint of deer was not available. Moreover, the densely wooded environment made herding and capture of deer impossible. Therefore, in accordance with the report of the AVMA panel on euthanasia for wild and feral animals,[7] all deer were euthanatized by gunshot to the neck or head by professional animal-control personnel. Animal-control personnel were skilled marksmen and used appropriate firearms and ammunition.

Postmortem examination—Age of 67 of the 116 deer was estimated by examination of tooth eruption and wear.[8] Postmortem examination included examination of palatine (oropharyngeal) tonsil, cranial lymph nodes (medial retropharyngeal, mandibular, and parotid), trachea, lungs, tracheobronchial and mediastinal lymph nodes, liver, kidneys, spleen, and hepatic and mesenteric lymph nodes. Tissues with gross lesions suggestive of tuberculosis were preserved in neutral-buffered 10% formalin and processed for microscopic analysis by use of routine paraffin embedment techniques. Sections were cut 5-µm thick and stained with H&E. Adjacent 5-µm sections were cut from blocks containing lesions suggestive of tuberculosis, stained by use of the Ziehl-Neelsen technique,[9] and examined thoroughly for acid-fast bacteria at 40× magnification. Results were considered positive when lesions morphologically consistent with tuberculosis contained acid-fast bacteria.

Bacteriologic culture—Specimens for bacteriologic culture were collected from all 116 deer. Specimens from tissues with gross lesions suggestive of tuberculosis were collected separately, whereas specimens from other tissues were pooled as follows: head (palatine [oropharyngeal] tonsil and medial retropharyngeal, mandibular, and parotid lymph nodes), thorax (lung and tracheobronchial and mediastinal lymph nodes), and abdomen (mesenteric and hepatic lymph nodes). Specimens were placed in sterile bags and frozen at –20 C for shipment and transferred to –80 C for storage until processing. Processing of specimens was performed as described.[10] Results were considered positive when *M bovis* was isolated.

Tonsillar, nasal, oral, and rectal swab specimens and fecal samples were also collected from all deer for bacteriologic culture. Swab specimens were collected with an 18-cm cytology brush[b] and processed as described.[11]

Environmental samples, including hay and pelleted feed from 13 of 16 feeding sites, soil around feeding sites, and water from 2 natural ponds used as watering sites, were collected in March and November 1998 for bacteriologic culture. Pelleted feed, soil, water, and fecal samples were processed by hand mixing 5 g of sample with 50 ml of phenol red broth in a 50-ml centrifuge tube. The mixed sample was allowed to settle for 30 minutes. Twenty milliliters of supernatant was removed, placed in a new 50-ml centrifuge tube, and incubated overnight at 37 C. After incubation, an equal volume of 1.0 *N* NaOH was added for decontamination. After 20 minutes, the sample was centrifuged at 2,000 × g for 20 minutes. The supernatant was decanted and the sediment suspended in 1 ml of **phosphate-buffered saline (PBS)** solution with 50 µg of amphotericin B/ml. One hundred microliters of the sample was inoculated onto separate agar slants containing Stonebrink medium, Herrold egg yolk medium, and Middlebrook 7H10 and Middlebrook 7H11 media. Inoculated agar slants were incubated at 37 C for 8 weeks. Two hundred microliters of each sample were also inoculated into vials of commercial media[c] containing selected antimicrobials[d] for isolation of mycobacteria. Inoculated vials were processed as directed by the manufacturer.

Hay samples were processed by grinding 5 g of hay in a grinder with 100 ml of phenol red broth. The mixed sample was allowed to settle for 30 minutes, then 50-ml was removed, transferred to a new 50-ml centrifuge tube, and incubated for 4 hours at 37 C. Twenty-five milliliters of sample was added to 25 ml of 1.0 *N* NaOH. After incubation for 10 minutes, the pH of the sample was neutralized by addition of 10.0 *N* HCl. The sample was centrifuged for 20 minutes at 2,000 × g, the supernatant decanted, and the sediment suspended in 1 ml of PBS solution with 50 µg of amphotericin B/ml. Agar slants and commercial media vials[c] were inoculated and incubated as described for pelleted feed, soil, water, and fecal samples.

Statistical analyses—Ages of deer were expressed as mean years ± SEM. Mean age of tuberculous deer was compared with mean age of all deer removed from the premises by use of a Student *t*-test. Differences were considered significant at $P < 0.05$.

Results

Mycobacterium bovis was isolated from 14 of 116 (12%) deer. Nine of the 14 deer had gross or microscopic lesions consistent with tuberculosis. Most commonly affected tissues included the medial retropharyngeal lymph node (n = 6) and lung (4). Lesions were also detected in the tracheobronchial lymph node (n = 2), mediastinal lymph node (2), hepatic lymph node (2), mesenteric lymph node (2), small intestine (1), and palatine (oropharyngeal) tonsil (1). Five of the 14 deer had no gross lesions suggestive of tuberculosis; however, *M bovis* was isolated from pooled cranial lymph nodes and tonsils from these deer.

Bacteriologic culture of swab specimens of the tonsillar crypt region from 2 of the 14 infected deer yielded *M bovis*. Bacteriologic culture of a swab specimen from the tracheal lumen of 1 of these 2 deer also yielded *M bovis*. *Mycobacterium bovis* was not isolated from feces or rectal, nasal, or oral swab specimens from any deer. Likewise, *M bovis* was not isolated from any environmental sample.

The youngest tuberculous deer was approximately 6 months old. This particular deer was part of a group of 10 female fawns that had been hand raised after being separated from their dams at 1 to 3 days of age. These hand-raised fawns were to become part of an artificial insemination program. The fawn had tuberculous lesions in numerous tissues (lung, palatine tonsil, and medial retropharyngeal and hepatic lymph nodes). The remaining 9 fawns were free of tuberculosis.

The mean age of tuberculous deer was 2.5 ± 0.3 years (median, 2.5 years; range, 0.5 to 6 years; **Table 1**). The mean age of all deer removed from the premises was 2.5 ± 0.1 years (median, 2.5 years; range, 0.5 to 6 years). Difference in ages between these 2 groups was not significant. Seventy of the 116 (60%) deer were female, and 46 (40%) were male; 9 of 14 (64%) tuberculous deer were female, and 5 (36%) were male.

One coyote (*Canis latrans*) was killed during the

Table 1—Age of 67 deer with and without evidence of infection with *Mycobacterium bovis*

	Tuberculous deer			All deer		
Age	**Female**	**Male**	**Total***	**Female**	**Male**	**Total†**
< 12 months	1	0	1 (7)	2	2	4 (6)
13–24 months	2	1	3 (21)	11	5	16 (24)
25–48 months	5	4	9 (64)	27	17	44 (66)
> 48 months	1	0	1 (7)	3	0	3 (4)
Total	**9**	**5**	**14 (100)**	**43**	**24**	**67 (100)**

*Number in parentheses represents percentage of all tuberculous deer. †Number in parentheses represents percentage of all deer examined.

deer removal effort. The coyote was found to be in excellent body condition with no gross or microscopic lesions consistent with tuberculosis. However, *M bovis* was isolated from pooled-head and thoracic-tissue specimens.

Discussion

Surveys of hunter-killed deer for tuberculosis in Michigan and other states routinely involve submission of deer heads by hunters to state animal health officials where the cranial lymph nodes are examined for gross lesions consistent with tuberculosis.[1,12] In this population of captive deer, such an examination would have identified only 6 of 14 (43%) tuberculous deer. Gross examination of the head and lungs (with associated lymph nodes) would have identified 9 of 14 (64%) tuberculous deer. These findings suggest that surveys involving examination of the head alone may underestimate the prevalence of disease by as much as 57%. However, more accurate results achieved by submission of head and lungs for examination must be weighed against increased cost and possible decreased hunter compliance.

Similar to findings in red deer (*Cervus elaphus*),[13] the medial retropharyngeal lymph node was a common site for tuberculous lesions. However, unlike red deer, the white-tailed deer in this study did not commonly have lesions in the palatine (oropharyngeal) tonsil.[13] Although only 1 of the white-tailed deer in the present study had tuberculous lesions in the tonsil, *M bovis* was recovered from tonsillar crypt swab specimens from 2 additional deer. Moreover, bacteriologic culture of pooled head samples from 5 deer, which included the tonsil, yielded *M bovis*. Tonsillar lesions have also been detected in cattle with naturally occurring tuberculosis.[14] The results of the present study support the hypothesis that the tonsil and associated lymph nodes play an important role in *M bovis* infection in animals.[13,14] Unlike red deer, suppurating, draining sinuses from infected cranial lymph nodes were not a feature in any of the white-tailed deer examined in the present study.[13] Such lesions would represent a likely means of disease transmission to other animals.

The finding of disseminated tuberculosis in a young fawn was surprising. Infection at this early age suggests transmission from doe to fawn through the placenta, milk, or close contact and grooming. Shedding of mycobacteria from the mammary gland is well-documented in cattle and goats and is thought to occur late in the course of disease.[15,16] In experimentally infected white-tailed deer, *M bovis* is excreted in nasal and oral secretions.[11] It is plausible that *M bovis* may be transmitted through infected fluids during grooming or other interactions between dam and fawn. In a model of disease transmission applied to the tuberculosis epidemic in Michigan white-tailed deer, maternal transmission was determined to be unimportant in maintaining disease.[17] Our results suggest that maternal transmission of *M bovis* can occur naturally in white-tailed deer. Naturally occurring tuberculosis has also been diagnosed in a 5-month-old red deer fawn from New Zealand. That fawn, euthanatized during a tuberculosis surveillance study, had a single lesion in the hepatic lymph node.[18]

The ratio of females to males among our tuberculous deer reflects the ratio of females to males among all deer removed, suggesting that tuberculosis was equally likely to develop in males as females. However, caution should be exercised in reaching any conclusion concerning sex predilection with only 14 affected animals. Previous reports of tuberculosis in white-tailed deer[1] and red deer[13] found no evidence that sex predilection exists.

The mean, median, and range of ages of tuberculous deer in this study were similar to ages described in the initial report of the outbreak of tuberculosis in wild white-tailed deer in Michigan (2.7, 2.5, and 1.5 to 5.5 years, respectively).[1] This suggests that ages of the deer examined in this captive herd were similar to ages of wild deer harvested during the deer hunting season in Michigan. In a survey of tuberculosis in red deer, mature deer (24 to 48 months old) and aged deer (> 48 months old) were most commonly infected with *M bovis*.[13] The paucity of aged deer that we examined in this captive herd is likely attributable to selective removal of older males through hunting conducted prior to the study and selective removal of older does through culling practices of the owner. Older deer dying of tuberculosis or other natural causes was not a factor in this herd.

The isolation of *M bovis* from a coyote without evidence of tuberculous lesions is consistent with reported cases of *M bovis* infection in coyotes in North America.[19] It is hypothesized that coyotes are exposed to *M bovis* while feeding on carcasses of tuberculous deer. The lack of gross lesions was thought to be attributable to recent infection (lack of sufficient time for lesion development) or relative resistance of coyotes to tuberculosis.[19]

Lack of isolation of *M bovis* from environmental samples, including feed and water, may have been a result of low numbers of mycobacteria in a large volume of sample, too few samples collected, or lack of ability of *M bovis* to survive in the environment. Survivability of *M bovis* in the environment is highly variable and dependent on temperature, humidity, and exposure to direct sunlight.[20] Depending on the conditions, *M bovis* may survive in soil from 18 to 332 days.[20-23] Evidence suggests that tuberculous wildlife may contaminate feed intended for livestock, and *M bovis* may survive long enough on feed to serve as a source of infection to domestic livestock.[24]

[a]Whipple DL. DNA fingerprinting of *Mycobacterium bovis* isolates from a cow, five coyotes, and two raccoons originating from northeastern Michigan (abstr), in *Proceedings*. US Anim Health Assoc 1998;102:681.
[b]Cytobrush, Puritan Medical Products, Guilford, Me.
[c]BACTEC 12 B medium, Becton Dickinson Co, Sparks, Md.
[d]BACTEC PANTA PLUS, Becton Dickinson Co, Sparks, Md.

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