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Culture and Serologic Survey for *Mycobacterium avium* subsp. *paratuberculosis* Infection among Southeastern White-tailed Deer (*Odocoileus virginianus*)

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ABSTRACT: From July 1998 through October 2002, radiometric culture (ileocecal lymph node, mesenteric lymph node, and feces) and serologic testing by enzyme-linked immunosorbent assay (ELISA) were used to survey white-tailed deer (*Odocoileus virginianus*) from the southeastern United States for infection by *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*), the causative agent of paratuberculosis (Johne's disease). *Mycobacterium avium* subsp. *paratuberculosis* was isolated from the ileocecal lymph node of one of 313 deer (0.3%) originating from 63 populations in Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, and West Virginia (USA). Six deer (2%), all from different populations, had ELISA results above a 0.25 sample-to-positive cutoff value, but none of the ELISA reactors originated from the population from which the single *Mptb* isolation was made. These six deer were seronegative when tested by agar gel immunodiffusion (AGID). Collectively, these data indicate that white-tailed deer currently do not constitute a broad regional reservoir for *Mptb*; however, further study is warranted to clarify the significance, if any, of infected deer to the epizootiology of paratuberculosis on a local scale. Adaptation and validation of an ELISA or another serologic assay for use with deer and other wildlife would markedly enhance *Mptb* surveillance among wild populations and would be a powerful tool for gaining information on the role of wild species in epidemiology of paratuberculosis.

Key words: ELISA, Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis*, *Odocoileus virginianus*, radiometric culture, survey, United States, white-tailed deer.

Paratuberculosis, or Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*), occurs worldwide and is recognized as a significant health problem for domesticated ruminants, wild ruminants in zoologic collections, and farmed cervids (Williams, 2001). In the United States, a 1996 national survey found that 22% of dairy cattle herds had at least a 10% infection rate and 41% of herds had at least one cow positive by enzyme-linked immunosorbent assay (ELISA) (Wells and Wagner, 2000). Infection with *Mptb* has been confirmed in multiple farmed red deer (*Cervus elaphus elaphus*) herds in the United States and in more than 300 red deer operations in New Zealand (Mackintosh, 2002).

Compared with traditional livestock or captive wild ruminants, reports of clinical or subclinical infection with *Mptb* among free-ranging wild ruminants are infrequent (Williams, 2001; Quist et al., 2002). Among free-ranging native wild ruminants in North America, clinical paratuberculosis has been reported in populations of Rocky Mountain bighorn sheep (*Ovis canadensis*; Williams et al., 1979), tule elk (*C. elaphus nannodes*; Jessup et al., 1981; Cook et al., 1997; Manning et al., 2003), Rocky Mountain elk (*C. elaphus nelsoni*; Manning, unpubl. data), and Key deer (*Odocoileus virginianus clavium*; Quist et al., 2002). Additionally, *Mptb* was isolated from two of 10 clinically normal wild white-tailed deer from a cattle farm of known infection (Chiodini and Van Kruinningen, 1983). Both mule deer (*Odocoileus hemionus*) and white-tailed deer have been infected experimentally (Williams et al., 1983a, b). Worldwide, *Mptb* infection has been infrequently reported in nonruminant species as well, including, for example, primates (Zwick et al., 2002) and rabbits (Beard et al., 2001).

Although it is clear that free-ranging wild ruminants are susceptible to infection, in North America, the infection apparently is maintained within only a few wild populations (Williams, 2001). A better understanding of the prevalence, distribution, and ecology of paratuberculosis within wild ruminant populations is desirable from a wildlife health perspective. Furthermore, this epidemiologic information would be important in the development of a national paratuberculosis control program for livestock. Here, we report a culture and serologic survey for *Mptb* infection among white-tailed deer populations in the southeastern United States.

White-tailed deer in this study were collected for population health evaluations by the Southeastern Cooperative Wildlife Disease Study (SCWDS, Athens, Georgia, USA) on the request of state or federal natural resource management agencies from July 1998 through October 2002. These health evaluations involved necropsy and laboratory testing of five adult animals from each of 63 deer populations in Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, and West Virginia (USA). Two exceptions were six deer tested from one population in Mississippi and two deer tested at one location in South Carolina. Animals were collected by cervical gunshot, temporarily chilled for 6–12 hr, and necropsied within 8–18 hr of death. At necropsy, the ileocecal lymph node, an approximately 3-cm segment of mesenteric lymph node, and about 10–25 g of feces per rectum were collected, placed in separate plastic bags (Whirl-Pak®, Fisher Scientific, Suwanee, Georgia, USA), stored on cold packs, and shipped by overnight courier to the Johne's Testing Center (School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, USA). Culture for *Mptb* was conducted on each of these samples with a radiometric (BACTEC) technique, and the identities of mycobacterial isolates were confirmed by IS*900* DNA polymerase chain reaction probe, mycobactin-dependent growth patterns, and high-performance liquid chromatography (HPLC) analyses of mycobacterial mycolic acids. Culture and identification methods used in this study previously have been described in detail (Collins et al., 1990; Quist et al., 2002). Acid-fast isolates determined not to be *Mptb* according to morphology, lack of mycobactin dependency, and absence of IS*900* were identified by HPLC methods (Butler et al., 1991).

Immediately following death, blood was collected from each animal by cardiac puncture, placed in a 50-ml tube and allowed to clot; serum was harvested within 12 hr and stored at −20 C for serologic testing. Sera were tested by a commercially available ELISA assay (IDEXX, Portland, Maine, USA). As per kit instructions for cattle and in accord with a previous study of Key deer (Quist et al., 2002), an ELISA sample-to-positive (S/P) value of 0.25 was chosen as the cutoff value to discriminate test-positive sera from test-negative sera, although the S/P value yielding the best sensitivity and specificity rates for this assay in white-tailed deer is not known. Sera with S/P>0.25 were also tested by agar gel immunodiffusion (AGID; Immunocell, Portland, Maine, USA).

Cultures were successfully completed on 930 of 939 (99%) samples from 313 deer; nine samples (1%) were lost to contamination. All three samples were completed for 306 deer; among the remaining seven deer, cultures were successfully completed on both lymph nodes for three deer, one lymph node and feces for two deer, and feces only for two deer. *Mycobacterium avium* subsp. *paratuberculosis* was isolated from the ileocecal lymph node of one deer from Kentucky, from which all three samples were cultured to completion (Table 1). Two other species of mycobacteria—one isolate of *Mycobacterium abscessus* and two isolates of *Mycobacterium terrae* complex—were obtained

TABLE 1.. Radiometric culture isolation of *Mycobacterium* spp. from ileocecal lymph node, mesenteric lymph node, and feces of 313 white-tailed deer collected in the southeastern United States from July 1998 through October 2002.

State	No. tested		No. positive	Species isolated	Samples positive
	Populations	Deer			
Alabama	5	25	0	—	—
Arkansas	9	45	1	*M. terrae* complex	—
Florida	2	10	0	—	Feces
Georgia	6	30	0	—	—
Kentucky	5	25	1	*M. avium* subsp. *paratuberculosis*	—
Louisiana	1	5	0	—	Ileocecal node
Maryland	3	15	0	—	—
Mississippi	4	21	0	—	—
North Carolina	8	40	0	—	—
South Carolina	4	17	0	—	—
Tennessee	6	30	1	*M. terrae* complex	Ileocecal node
West Virginia	10	50	1	*M. abscessus*	Feces

from deer originating in Arkansas, Tennessee, and West Virginia. Mycobacteria were isolated from a single sample for each of these four deer, and gross lesions suggestive of mycobacterial infection were not noted in any of the deer from which mycobacterial isolates were obtained.

Serum samples from 307 (98%) deer produced S/P≤0.25 by ELISA (Fig. 1), whereas the remaining six deer (2%) had S/P values above 0.25 (viz., 0.34, 0.39, 0.39, 0.44, 0.83, 1.65). The six ELISA-reactive deer were from five populations, but none were from the population from which the *Mptb* isolate was obtained, including the culture-positive animal. All six of the samples with ELISA values above 0.25 were negative when tested by AGID. The negative AGID results might indicate that the antibody detected by the ELISA was elicited by an infection with a pathogen other than *Mptb* because AGID, although considered less sensitive, is regarded as a more specific assay in some species (Shulaw et al., 1993). The overall ELISA results suggest a low prevalence of any mycobacterial infections among the deer examined.

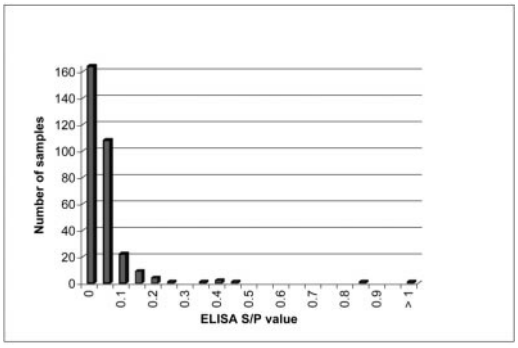

FIGURE 1. Distribution of enzyme-linked immunosorbent assay (ELISA) sample-to-positive (S/P) values for 313 white-tailed deer from the southeastern United States tested for antibodies to *Mycobacterium avium* subsp. *paratuberculosis.* A cutoff value of S/P=0.25 was used to discriminate reactive from nonreactive serum samples.

A major impetus for this study was recognition that paratuberculosis might become the subject of a national control program similar to industry-state-federal eradication/control programs for other major livestock diseases. In this larger context, or even in the context of control within domestic animal herds absent implementation of a national paratuberculosis program, risk of infection from potential wild animal reservoirs is a critical factor in developing an effective disease management strategy. The low prevalence of *Mptb* infection among white-tailed deer in this

study is in agreement with the concept that paratuberculosis currently is rare among wild ruminants in North America, with the exception of a few focal populations (Williams, 2001). Specifically, our data indicate that wild white-tailed deer do not constitute a broad regional reservoir for *Mptb*; thus, infected deer would not appear to be a pivotal factor in any future industry-state-federal paratuberculosis eradication/control program.

The single culture-positive deer was collected in August 2000 from Kleber Wildlife Management Area (WMA, Owen County, Kentucky), but whether this culture-positive deer reflected independent maintenance of *Mptb* within wild deer or acquisition from a domestic animal source is unknown. In each prior instance in which *Mptb* infection was confirmed among North American wild ruminant populations, potential risk factors were described, including high population density (Cook et al., 1997; Jessup and Williams, 1999; Quist et al., 2002), gregarious behavior and repeated use of traditional bedding areas (Jessup and Williams, 1999), concentration at supplemental feeding sites (Quist et al., 2002), and presence of infected cattle (Chiodini and Van Kruinningen, 1983). A portion of Kleber WMA had been a dairy farm prior to its acquisition by the Kentucky Department of Wildlife and Fisheries in 1962, and dairy and beef cattle farms currently exist in the immediate vicinity of Kleber WMA. Of the risk factors noted above, the only one potentially present at Kleber WMA is a domestic ruminant source. In the two prior instances of confirmed paratuberculosis among wild white-tailed deer, cattle were a probable source for a local Connecticut deer population (Chiodini and Van Kruinningen, 1983), but a domestic animal or other extrinsic source was not identified for an insular Key deer population (Quist et al., 2002).

Although culture surveys to estimate infection rates have been conducted on a few local wild ruminant populations (bighorn sheep, tule elk, and Key deer) known or suspected to independently sustain *Mptb* infection (Williams et al., 1979; Cook et al., 1997; Quist et al., 2002), larger scale surveys for *Mptb* are lacking for other native North American wild ruminants. As potential for exposure among wildlife increases because of shared range or contact with infected domestic species, it will become important to monitor the spread, if any, of the infection into wildlife and the possibility of its return to domestic species (Daniels et al., 2003; Deutz et al., 2003).

When choosing population screening methods, one must take into account expected prevalence, sample collection and processing requirements, the sensitivity of the assays, and cost. Detection of *Mptb* infection in a single survey for low-prevalence herds is difficult by any method, and serial testing with multiple assays might be necessary to obtain the most accurate assessment of prevalence. Culture, microscopic, or both types of examination of extraintestinal tissue is a critical component of surveillance and is necessary to rule out the possibility that an isolation of *Mptb* from a fecal sample was a result of "pass-through" from a contaminated environment vs. true infection. Preferred samples for isolation are mesenteric and ileocecal lymph nodes and ileum. Assays of tissues from animals with clinical signs compatible with Johne's disease (such as poor body condition or diarrhea) are likely to be more rewarding than from apparently healthy animals.

Fecal sample pooling can be used to reduce the overall cost of culture surveillance but is less sensitive than individual sample testing (Sergeant et al., 2002; Wells et al., 2002; Schroen et al., 2003). The sensitivity of pooled fecal samples specifically in free-ranging wildlife has not been evaluated. Freezing of samples should be avoided to maintain the viability of as many organisms as possible (Richards, 1977).

Serum is a standard sample for wildlife health surveillance (assays can be per-

formed for antibodies to many pathogens, storage is possible for long periods with minimal degradation of the antibody, and rarely is contamination at collection a problem). Testing by ELISA can be a fast and inexpensive first-step method to "triage" wildlife populations into probable infected vs. uninfected categories; however, the current ELISA has not been fully validated in deer, making interpretation of results somewhat speculative (Shulaw et al., 1986; Williams, 2001; Quist et al., 2002). An ELISA with a cervid-specific conjugate or protein G conjugate should be used for populations of deer, and interpretation should be done on a herd rather than on an individual animal basis (Collins, 1996; Kramsky et al., 2003). Because of its low sensitivity, the AGID is not recommended for use with deer (Schroen et al., 2003). Ultimately, an array of validated assays potentially could establish *Mptb* status for populations as a whole, indicate the probable prevalence of infection within populations, and provide the basis for confirmatory culture-based surveillance to those populations with the greatest likelihood of *Mptb* infection.

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