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Tuberculin skin testing in white-tailed deer (*Odocoileus virginianus*)

Mitchell V. Palmer, Diana L. Whipple, W. Ray Waters

Abstract. The comparative cervical skin test for antemortem diagnosis of tuberculosis was done 169 times on 116 different white-tailed deer of known *Mycobacterium bovis* infection status. The sensitivity and specificity were 97 and 81%, respectively. The magnitude of change in skin thickness at test sites was not significantly influenced by dosage of inoculum, dissemination of the disease process, or repeated skin testing. However, the magnitude of change in skin thickness was significantly greater in deer infected for less than 109 days than in deer infected for more than 109 days. As used in the present study, the comparative cervical skin test is a sensitive method of antemortem diagnosis of *M. bovis* infection in white-tailed deer.

The captive Cervidae industry has grown remarkably in the United States over the last 20 years. The North American Deer Farmers organization has over 400 active producer members that commercially raise over 75,000 head of fallow (*Dama dama*), axis (*Axis axis*), red (*Cervus elaphus*), white-tailed (*Odocoileus virgninianus*), or sika deer (*Cervus nippon*). The North American Elk Breeders Association, with over 1,700 members, estimates that there are over 150,000 farmed elk (*Cervus elaphus*) in the United States.

Tuberculosis of captive Cervidae became an important disease in the United States in 1990 and 1991, when investigations were prompted by the identification of a tuberculous elk in Canada that had been imported from the United States in 1988. Testing of domestic herds with links to the Canadian elk revealed tuberculosis in 10 different elk herds in 8 different states.[2] The discovery of tuberculosis in captive elk in the United States resulted in the addition of captive Cervidae to the US Department of agriculture (USDA) uniform methods and rules (UMR) for the eradication of bovine tuberculosis.[10] The inclusion of regulations

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for the eradication of tuberculosis in captive Cervidae to the UMR is justified because it has been documented that captive Cervidae can represent a source of infection for domestic cattle.[3]

Antemortem diagnosis of tuberculosis in animals has relied on measurement of delayed-type hypersensitivity (DTH) by intradermal injection of mycobacterial extracts, the most common of which is purified protein derivative (PPD). Early investigation of intradermal skin testing of Cervidae showed the caudal fold test, as performed in cattle, to be unreliable in Cervidae.[9] Cervical skin testing, by injection of *M. bovis* PPD in the skin of the lateral neck, was found to be more reliable than caudal fold skin testing. Currently, only intradermal skin testing and the blood tuberculosis (BTB) test are approved by the USDA for antemortem diagnosis of tuberculosis in Cervidae.[10] Currently, however, the BTB test is not available in the United States.

Typically, Cervidae in a herd of unknown infection status are tested by the single cervical tuberculin test (SCT) and the results are categorized as negative, suspect, or reactor. Suspects, as identified by the SCT, may be reevaluated using the comparative cervical tuberculin test (CCT). The CCT involves injection of PPD from *M. bovis* (bovine PPD) and *Mycobacterium avium* (avian PPD) at 2 different sites on the lateral neck. The addition of the avian PPD in the CCT increases specificity as compared with the SCT. Animals infected with atypical mycobacteria may be suspects or reactors on the SCT; however, using the CCT, these animals can often be identified due to a greater change in skin thickness at the avian PPD injection site compared with the change in skin thickness at the bovine PPD injection site. Results of the CCT are then used to categorize deer as negative, suspect, or reactor.

Little information exists on the accuracy of intradermal skin testing for tuberculosis in individual species of Cervidae. In New Zealand, the sensitivity of the SCT in naturally or experimentally infected red deer has been reported to be 82 and 86%, respectively.[1,4] Specificity has varied from 46 to 76% in herds of tuberculosis-negative red deer.[9] In a study limited to experimentally infected red deer, the CCT had a sensitivity and specificity of 91.4 and 98.7%, respectively.[9] In a separate evaluation of the CCT using naturally infected fallow and sika deer, the sensitivity and specificity were found to be 84 and 80%, respectively.[5] In the United States, the specificity of the SCT in all Cervidae is between 71 and 86%.[9] The sensitivity is reported to be 100%; however, SCT-negative animals were not euthanized and examined; thus, the true infection status of these animals is not known.[9] In the United States, where numerous species of Cervidae are raised for commercial purposes, few studies have investigated the accuracy of intradermal tuberculin testing in selected species of deer.

One hundred sixty-nine intradermal skin tests (CCT) were conducted on 116 white-tailed deer of known infection status. Sixty deer were confirmed infected with *M. bovis* through thorough postmortem examination and bacteriologic culture of tissues. Forty-nine of the known infected animals were part of tuberculosis pathogenesis studies and had been experimentally infected, as described,[8] with various dosages of *M. bovis*, ranging from 300 colony-forming units (CFU) to 2×10^8 CFU. Eleven deer were part of a tuberculosis transmission study and had been infected through contact exposure to experimentally infected deer. *Mycobacterium bovis* strain 1315, originally isolated from a white-tailed deer in Michigan, was used for all experimental infections.

The CCT was done as described in USDA Animal and Plant Health Inspection Service, Veterinary Services guidelines.[10] Hair was clipped from 2 sites on 1 side of the midcervical region and the skin thickness of each site was measured. One tenth milliliter avian PPD (0.4 mg/ml) was injected intradermally in the uppermost site and 0.1 ml of bovine PPD (1 mg/ml) was injected into the lower site. Injection sites were observed, palpated, and measured 72 hours after injection. Results were interpreted by plotting measurements on a graph (VS from 6-22D) developed by the USDA for interpretation of the CCT for Cervidae. Based on the results, animals were categorized as negative, suspect, or reactor. In addition to change in skin thickness at the respective injection sites, other information recorded included dosage of inoculum, classification of the resulting disease as disseminated or localized, number of tissue sites affected, duration of infection, and isolation of atypical mycobacteria. Affected tissues were those with gross or microscopic lesions consistent with tuberculosis or tissues from which *M. bovis* was isolated. As previously described, lesion distribution was characterized as localized (gross or microscopic lesions in 1 or more of the following sites: tonsils, oropharyngeal lymph nodes, thoracic lymph nodes, lung) or disseminated (gross or microscopic lesions in sites in addition to those seen in localized disease).[7] Means of parameters of interest were compared using the Student's *t*-test. Differences were considered statistically significant when $P < 0.05$.

One hundred sixty-nine tests were done on 116 deer. Thirty-eight deer were tested twice, while 15 deer were tested 3 times. Repeat tests were separated by a minimum of 90 days. Of 169 tests, 60 were done in known *M. bovis*-infected deer, while 109 were done in known noninfected deer. Of the 60 known *M. bovis*-infected deer, the CCT identified 58 (97%) as reactors and 2

(3%) as suspect. No *M. bovis*-infected deer were categorized as negative. The CCT identified 88 (81%) noninfected deer as negative, 15 (14%) as suspect, and 6 (5%) as reactors.

As expected, the change in skin thickness at the bovine PPD injection site was significantly greater in *M. bovis*-infected deer than that seen in noninfected deer (Table 1). While there was also a significant difference in change in skin thickness between *M. bovis*-infected deer and noninfected deer at the avian PPD injection site, this difference was not sufficient to change the categorization of the deer as reactor, suspect, or negative on the USDA graph. *Mycobacterium avium* or other atypical mycobacteria were isolated from 3 deer. The presence of atypical mycobacteria did cause the DTH response to be larger at the avian PPD injection site when compared with deer from which no mycobacteria were isolated; however, this response was not statistically significant (Table 1). In 1 case, *M. gastri* was isolated from a non-*M. bovis*-infected deer categorized as a reactor by the CCT.

In all experimentally infected deer, the duration of infection could be precisely determined and ranged from 90 to 365 days (mean = 137, median = 100). Mean change in skin thickness at the bovine PPD injection site was significantly greater in deer that had been infected less than 109 days than those deer infected for greater than 109 days (Table 1). Dissemination of the disease process was evaluated in 41 of 60 *M. bovis*-infected deer. There was no significant difference in change in skin thickness at either the bovine or avian PPD injection sites when deer with disseminated tuberculosis ($n = 24$) were compared with deer with localized tuberculosis ($n = 17$).

There was no significant difference in the change in skin thickness at the bovine or avian PPD injection sites between deer receiving high ($>10^3$ CFU) or low ($\leq 10^3$ CFU) dosages of *M. bovis* (Table 1). Repeated testing of *M. bovis*-infected or noninfected deer did not significantly alter the magnitude of the change in skin thickness at the bovine PPD or avian PPD injection sites or change the resulting categorization of the response.

In the present study, the magnitude of the change in skin thickness at the site of intradermal tuberculin skin testing was not influenced by dosage of inoculum, dissemination of the disease process, or repeated skin testing. All of the infected deer used in the present study were experimentally infected with *M. bovis* or were infected through contact with experimentally infected deer. It is possible that results would vary in naturally infected white-tailed deer.

Previous experiments in cattle and deer have shown that, as *M. bovis* infection progresses, the immune response is altered from one of primarily cell-mediated

Table 1. Change in skin thickness (mm) ± standard error of white-tailed deer tested for exposure to *M. bovis* by the comparative cervical skin.

PPD*	Noninfected ($n = 109$)	Experimental *M. bovis* infection ($n = 60$)	Atypical mycobacteria† isolated ($n = 3$)	Disseminated disease‡ ($n = 24$)	Localized disease‡ ($n = 17$)	Dose $\leq 10^3$ CFU§ ($n = 29$)	Dose $>10^3$ CFU ($n = 20$)	Duration of infection ≤ 109 days ($n = 32$)	Duration of infection >109 days ($n = 28$)
M. bovis	0.81 ± 0.2	12.1 ± 0.7	6.2 ± 4.9	10.2 ± 1.2	11.2 ± 0.9	14.3 ± 0.8	11.5 ± 1.3	13.7 ± 0.8	10.3 ± 1.1
M. avium	1.2 ± 0.2	4.0 ± 0.4	9.0 ± 2.6	4.2 ± 0.7	3.4 ± 0.4	4.3 ± 0.5	4.5 ± 0.7	4.7 ± 0.5	3.3 ± 0.6

* PPD = purified protein derivative.
† Environmental or other non-*M. bovis* mycobacteria.
‡ See text for definition.
§ CFU = colony-forming units.

immunity to one of primarily antibody production.[6] This results in diminution of delayed-type hypersensitivity and an increase in serum antibody titers to various *M. bovis* antigens.[10] In the present study, the cell-mediated response, as measured by intradermal skin testing, did not decrease as tuberculosis progressed to a disseminated form. However, as the duration of infection increased, the size of the reaction at the bovine PPD injection site decreased, suggesting a change in the cell-mediated immune response. Other assays to measure cell-mediated or humoral immunity were not considered in the present study.

In the current report, the CCT was not preceded by the SCT, as described in the UMR for the eradication of bovine tuberculosis. However, in testing captive wildlife, when repeated handling, restraint, and risk of injury to animals and personnel are problematic, it may be appropriate, although not officially recommended, to utilize the CCT as the initial screening test. In spite of the technical difficulties associated with intradermal tuberculin skin testing of Cervidae, the CCT remains a sensitive and moderately specific means of antemortem diagnosis of tuberculosis in white-tailed deer. Although the ideal test is one with high sensitivity and specificity, in disease eradication schemes, it may be appropriate to accept tests with high sensitivity and only moderate specificity. In such a scenario, some false-positive results and condemnation of uninfected animals are tolerated while confidence is maintained that few, if any, false-negative animals remain behind to cause reinfection in tested herds.

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