Humoral Immune Responses of White-Tailed Deer (*Odocoileus virginianus*) to *Mycobacterium bovis* BCG Vaccination and Experimental Challenge with *M. bovis*

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Humoral Immune Responses of White-Tailed Deer
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Monitoring of the kinetics of production of serum antibodies to multiple mycobacterial antigens can be useful as a diagnostic tool for the detection of Mycobacterium bovis infection as well as for the characterization of disease progression and the efficacy of intervention strategies in several species. The humoral immune responses to multiple M. bovis antigens by white-tailed deer vaccinated with BCG orally via a lipid-formulated bait (n = 5), orally in liquid form (n = 5), and subcutaneously (n = 6) were evaluated over time after vaccination and after experimental challenge with virulent M. bovis and were compared to the responses by unvaccinated deer (n = 6). Antibody responses were evaluated by using a rapid test (RT), a multitarget immunoenzyme assay (MAPIA), a lipoarabinomannan enzyme-linked immunosorbent assay (LAM-ELISA), and immunoblotting to whole-cell sonicate and recombinant antigen MPB83. MAPIA and RT detected minimal to no antibody responses over those at the baseline to multiple M. bovis antigens in vaccinated white-tailed deer after challenge. This was in contrast to the presence of more readily detectable antibody responses in nonvaccinated deer with more advanced disease. The LAM-ELISA results indicated an overall decrease in the level of production of detectable antibodies against lipoarabinomannan-enriched mycobacterial antigen in vaccinated animals compared to that in nonvaccinated animals after challenge. Immunoblot data were inconsistent but did suggest the occurrence of unique antibody responses by certain vaccinated groups to Ag85 and HSP70. These findings support further research toward the improvement and potential use of antibody-based assays, such as MAPIA, RT, and LAM-ELISA, as tools for the antemortem assessment of disease progression in white-tailed deer in both experimental and field vaccine trials.

Free-ranging white-tailed deer (Odocoileus virginianus) are wildlife reservoirs for bovine tuberculosis (BTb), caused by Mycobacterium bovis, in the state of Michigan (15, 18). Current management strategies, such as population reduction and decreased supplemental feeding, have effectively reduced the prevalence of disease (15). However, BTb continues to maintain a low-level presence in the wild deer population. The inclusion of effective field vaccination as part of disease management efforts in deer herds with endemic BTb would significantly aid in efforts to eradicate BTb from this potential wildlife reservoir (14, 16).

Vaccination with M. bovis bacillus Calmette-Guérin (BCG) via the oral or the parenteral route is effective in protecting white-tailed deer from disease caused by experimental M. bovis infection (14, 16). An important component of the evaluation of any vaccine candidate is gaining an understanding of the dynamics of a recipient’s immunologic response to vaccination and infection over time in comparison with the dynamics of the responses in unvaccinated subjects (3, 19, 22). Previous research has shown that monitoring of the kinetics of production of serum antibodies to multiple mycobacterial antigens is useful for the characterization of disease progression and the efficacy of disease treatment and as a tool for the diagnosis of M. bovis or M. tuberculosis infection in several species (11, 12, 21, 23, 24, 25).

In the present study, the humoral immune responses to multiple M. bovis antigens by white-tailed deer vaccinated with BCG via the oral and the parenteral routes were evaluated by four different assays over time after vaccination and after experimental challenge with virulent M. bovis. This information provides an understanding of the differences in the immunologic responses and disease progression in vaccinated and unvaccinated white-tailed deer infected with M. bovis and insight regarding the appropriate diagnostic tests to be used for the detection of BTb in a vaccinated population.
MATERIALS AND METHODS

Deer, vaccination, challenge, and necropsy. Serum samples from 22 yearling white-tailed deer doves were utilized for this study. These animals were part of a larger herd obtained for a vaccine efficacy trial (14). The deer originated from four TB-free deer farms throughout the state of Iowa and were housed for the vaccination and infection studies conducted at the USDA/ARS National Animal Disease Center (NADC) in Ames, IA. All deer were housed and cared for according to the guidelines of the Association for Assessment and Accreditation for Laboratory Animal Care International. The Institutional Animal Care and Use Committee at the University of Otago approved animal protocols detailing the procedures and animal care prior to the initiation of the experiments.

At the beginning of the study, five deer voluntarily consumed 1 × 10⁶ CFU BCG Danish strain 1331 orally via a lipid-formulated bait (oral bait group) (1, 14); five deer received 1.9 × 10⁶ CFU BCG in culture medium orally via a catheter (oral liquid group), as described by Nol and others (14); six deer received 3.4 × 10⁵ CFU BCG subcutaneously in the right shoulder (parenteral vaccination group); and six deer received culture medium orally via a catheter and served as unvaccinated controls (nonvaccinated animals). Mycobacterium bovis BCG Danish strain 1331 in culture and in lipid-formulated pellets was prepared by Immune Solutions Ltd. at the University of Otago, Dunedin, New Zealand, as described by Aldwell and others (1). The vaccine doses were determined by standard enumeration techniques by serial dilution plate counting on Middlebrook 7H11 medium (Becton Dickinson, Cockeysville, MD). Before vaccination and on a monthly basis throughout the study, blood was collected via the jugular vein for serologic analysis of the antibody responses. Three months after vaccination, the deer were moved from an outdoor facility to a biosafety level 3 animal building. The animals were separated into rooms with three to four animals per room, and the vaccinated deer were conformed with the unvaccinated deer. All deer were then challenged with 228 CFU of M. bovis strain 9839 (NADC designation) by the intratonsillar route (114 CFU/tonsil). This strain was described by Lyashchenko et al. (12). Bovine culture filtrate (MBCF) was obtained from a field strain of M. bovis (strain 791/1376; Veterinary Sciences Division) cultured in synthetic Sauton’s medium for 21 days. Bovine protein purified derivative was produced by the Veterinary Laboratories Agency (Weybridge, Addlestone, United Kingdom).

The results of the MAPIA were evaluated visually to determine the presence or the absence of bands corresponding to the antigen on the strip. Selected MAPIA results also underwent semiquantitative densitometry analysis by use of the scanned strips and Scion Image, a public-domain imaging program from the U.S. National Institutes of Health (http://rsb.info.nih.gov/nih-image/). The densitometry software produced arbitrary values of the relative absorbance for the purposes of identifying trends among treatment groups. All densitometry values were recorded after subtraction of the densitometry readings taken from the prevaccination strips. The densitometry values obtained at 2 months for MBCF and 4 months for Epitope10 and 16/83 were used for comparisons among the treatment groups.

Immunoblot assay. The antibody responses of the deer were evaluated over time by immunoblot analysis with two sets of antigens: a whole-cell sonicate (WCS) of M. bovis strain 95-1315 and MPB83 (a kind gift from Jim McNair, AFBI, Belfast, United Kingdom). Mycobacterium bovis WCS was described by Waters et al. (23). Electrophoresis and immunoblot assays were performed by the procedures described by Waters and others (23, 25). Antigen wells were loaded with 12% (wt/vol) polyacrylamide gels. Electrophoretic transfer of the proteins onto pure nitrocellulose was accomplished with a Trans Blot cell (Bio-Rad, Hercules, CA) and sodium phosphate buffer (25 mM, pH 7.8) at 0.8 A for 90 min. After transfer, the filters were blocked with PBST and 2% (wt/vol) bovine serum albumin (BSA). After the filters were blocked, they were placed into the slot blot device and individual serum samples diluted 1:200 in PBST-BSA were added to independent slots. After 2 h of incubation at room temperature with gentle rocking, the blots were washed three times with PBST and incubated with horseradish peroxidase-conjugated anti-goat immunoglobulin G heavy and light chains (Kirkegaard & Perry Laboratories) diluted 1:500 and then received another washing step. Deer antibodies bound to printed antigens were visualized with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted 1:500 and then received another washing step. Deer antibodies bound to printed antigens were visualized with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted 1:500 and then received another washing step. Deer antibodies bound to printed antigens were visualized with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted 1:500 and then received another washing step. Deer antibodies bound to printed antigens were visualized with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted 1:500 and then received another washing step.
control sera were diluted 1:100 in PBS containing 0.1% gelatin. After incubation overnight at 4°C with the diluted test sera, the wells were washed nine times with 200 µl/well PBST and the plates were incubated for 1 h at 37°C with 100 µl/well horseradish peroxidase-conjugated protein G from a *Streptococcus* sp. (strain P6170; Sigma) diluted 1:2,000 in PBS with 0.1% fish gelatin. The wells were washed nine times with 200 µl/well PBST, and the plates were incubated at room temperature with 100 µl/well SureBlue reagent (Kirkegaard & Perry Laboratories). The plates were read kinetically every minute for 15 min at 650 nm by using an automated enzyme-linked immunosorbent assay (ELISA) plate reader (FlexStation 3; Molecular Devices, Sunnyvale, CA). For each well, the maximum rate for the enzyme (slope of the bell curve) (slope of the bell curve) was determined by subtracting the *V* max value for the no-antigen well from the *V* max value for the well with antigen. The raw *W* max values were also calculated for the positive and the negative controls. Finally, the *V* max for the sample/in relation to a known positive sample was calculated by the following formula: (*V* max sample − *V* max negative control) (*V* max positive control − *V* max negative control). *V* max s/p was the normalized number for the maximum rate for change for the enzyme, and the value was used for data analysis. The mean *V* max s/p value for the pooled data for the vaccinated animals was compared to the mean *V* max s/p value for the nonvaccinated animals.

**Data analysis.** The number of vaccinated animals (pooled) with lesions was compared to the number of nonvaccinated animals with lesions by Fisher's exact test (two by two, one sided; Proc FREQ program of SAS software, version 9.1; SAS Institute, Cary, NC). Differences determined by the one-sided Fisher's exact test to have values less than or equal to 0.1 were considered significant. The total pathology scores derived from lymph node lesions, lung lesions, and lymph node and lung lesions combined for vaccinated and nonvaccinated animals were also compared by using the Kruskal-Wallis nonparametric one-way analysis of variance, followed by the Wilcoxon rank-sum test (SAS software, version 9.1; SAS Institute). Differences determined to be significant when *P* values were less than or equal to 0.1. Correlations between the relative densities obtained from the RT bands and pathology scores were analyzed by using Spearman's rank test (Proc FREQ; SCORR, program of SAS software, version 9.1; SAS Institute). When significant (*P* ≤ 0.1) effects were detected, Fisher’s protected least-significant-difference method was used to compare the data for the different treatment groups on specific sampling dates. A *P* value of ≤0.1 was considered significant.

### RESULTS

**Pathology.** Five of the 16 vaccinated animals developed lesions consistent with tuberculosis, but none of these animals received a total pathology score of greater than 3. All six nonvaccinated animals had lesions in one or more tissues, and the total pathology scores ranged from 5 to 30 (Table 1). The number of vaccinated deer with lesions was significantly lower than the number of nonvaccinated deer with lesions (Fisher’s exact test, *P* < 0.0002). The total lung pathology score, the total lymph node pathology score, and the lung and lymph node pathology scores combined were all significantly higher for the nonvaccinated group than for the vaccinated group (*P* = <0.001, *P* < 0.001, and *P* = 0.002, respectively, based on the Wilcoxon rank-sum test) (Fig. 1).

**RT.** Among the 16 vaccinated deer, only 1 animal developed detectable antibodies, as determined by RT, at any time during the study. One of the six deer in the parenteral vaccination group developed a response, as determined by RT, starting at 3 months postvaccination and remained reactive for 4 months after challenge. In contrast, five of six deer in the nonvaccinated group developed detectable antibodies, as determined by RT, by 4 months after *M. bovis* challenge. Two of those animals developed a response by 2 months postchallenge, and four deer did so by 3 months postchallenge. None of the nonvaccinated deer developed antibodies detectable by RT before *M. bovis* challenge. The relative densities obtained from the RT responses for all deer at 4 months postchallenge are summarized in Table 1. The relative densities of the RT responses in nonvaccinated deer at 4 months postchallenge were positively correlated (Spearman’s *R* = 0.60; *P* = 0.003) with the total combined pathology scores for the same group (Fig. 2). Interestingly, deer 19 and 24, the two animals in the nonvaccinated group with the lowest pathology scores, had the lowest densitometry readings in that group as well. However, these two deer still achieved higher pathology scores than any of the vaccinated deer (Table 1).

**MAPIA.** The following proteins were recognized by the animals in this study challenge, as determined by MAPIA, and are reported here in terms of the numbers of deer producing detectable antibodies to the following antigens after *M. bovis* challenge: 16/83 (n = 19), MBCF (n = 17), E6P10 (n = 5), CFP10 (n = 4), bovine protein purified derivative (n = 3), the
16-kDa protein (n = 2), MPB83 (n = 1), and ESAT-6 (n = 1). Although these data varied among both individuals and on the basis of the treatment group, three antigens (16/83, MBCF, and E6P10) were deemed the most useful for evaluation of the responses of vaccinated and nonvaccinated deer after M. bovis challenge. The relative densities measured from the responses of the deer in the individual treatment groups to 16/83 and E6P10 at 4 months postinfection and to MBCF at 2 months postinfection are summarized in Table 1. The overall numbers of vaccinated animals and nonvaccinated animals producing detectable antibodies to the three antigens over the 4-month postinfection period, as determined by MAPIA, are reported in Table 2.

Relative to the baseline levels (i.e., the preexisting responses, prior to vaccination), four of six deer in the nonvaccinated group had produced detectable antibodies to at least two of the three most useful antigens at 2 and at 4 months postinfection (Table 2). None of the 16 vaccinated deer produced detectable antibodies to more than one of the antigens throughout the postinfection period (Table 2). Six of the eight vaccinated animals that did produce detectable antibodies to one of the three most useful antigens had also done so before challenge (data not shown). In the oral bait vaccination group, zero of five, one of five, and three of five deer had detectable antibodies to E6P10 and 16/83 at 4 months postchallenge and to MBCF (2 months postchallenge), respectively (Table 1). One of five deer in the oral liquid vaccination group had detectable antibodies to E6P10 postchallenge. However, none of the deer in the oral liquid vaccination group had levels of antibodies to 16/83 or MBCF over the baseline levels at any time postinfection (Table 1). In the parenteral vaccination group, zero of six, one of six, and two of six deer responded to E6P10, 16/83, and MBCF, respectively (Table 1). In contrast, three of four, six of six, and five of six of the animals in the nonvaccinated group developed detectable antibodies to E6P10, 16/83, and MBCF, respectively (Tables 1 and 2).

**Immunoblots.** Specific bands of reactivity to M. bovis WCS at ~10 kDa, ~15 kDa, ~20 to 25 kDa, ~32 kDa, ~34 to 35 kDa, ~42 kDa, ~60 kDa, ~68 to 70 kDa, ~74 to 76 kDa, ~90 kDa, and ~125 kDa were detected by immunoblotting throughout the study. The most reactive proteins were at the ~20- to 25-kDa level, as all the deer produced antibodies to these antigens at some time during the study, including before vaccination.

Differences in antibody responses to proteins within specific size ranges were noted among the vaccination groups. During the first 3 months after M. bovis challenge, four of five animals in the oral bait vaccination group, two of five in the oral liquid vaccination group, five of six in the parenteral vaccination group, and five of six in the nonvaccinated group had responses to antigen at approximately the 32-kDa level greater than those detected at the baseline. However, by 4 months postchallenge, five of six nonvaccinated animals continued to produce antibody responses to these proteins, whereas two of five, two
of five, and three of six deer in the oral bait, oral liquid, and parenteral vaccination groups, respectively, maintained antibody responses (data not shown). Of the two deer in the oral bait vaccination group that maintained antibodies to the 32-kDa protein, one deer produced a very weak response. One of the two deer in the oral liquid vaccination group had a weak response, whereas the other deer (deer 84) had a very strong response and was the only deer in that group to have lesions. Unlike in the other vaccination groups, all three deer in the parenteral vaccination group produced strong antibody responses to the 32-kDa protein. Two of the three deer did not sustain lesions by the end of the study, and the third deer had a very low pathology score. The deer in the nonvaccinated group (deer 24) that did not respond to the 32-kDa protein was one of two deer that had the lowest pathology scores (score, 5). The other nonvaccinated deer with a low pathology score (deer 19) had only a weak response to the 32-kDa protein relative to the responses of the other four animals that responded.

All of the deer in the oral bait and the parenteral vaccination groups showed antibody responses to proteins at the 68- to 70-kDa level at some time in the 4 months postchallenge. At 4 months postchallenge, four of five of the deer in the oral bait vaccination group and four of six deer in the parenteral vaccination group still showed the production of antibodies to these proteins. Only one animal in each of the other two groups developed a response to these antigens. All of the deer in the parenteral vaccination group deer and two of the deer in the oral bait vaccination group had already developed antibodies to these proteins after vaccination.

Regarding the MPB83-specific immunoblot assay, there were no patterns in antibody response to this antigen among the vaccine groups. Three of five deer in the oral bait vaccination group became positive for this antigen at different time points after vaccination, of which only one developed an increased response at 1 month postchallenge, which persisted to 4 months postchallenge. None of the deer in the oral liquid vaccination group developed antibodies to MPB83 detectable by immunoblotting after vaccination or challenge; however, the prevaccination serum of one of the animals did have a response, but the animals was negative by every test thereafter. One of five deer in the parenteral vaccination group became weakly positive for MPB83 at 1 month postvaccination, and this response disappeared by 2 weeks after challenge. One of six deer in the nonvaccinated group developed detectable antibodies to MPB83 by immunoblotting 2 weeks after challenge and maintained this response until 4 months postchallenge.

**DISCUSSION**

Our MAPIA, RT, and LAM-ELISA results indicate that vaccinated white-tailed deer, which developed fewer and less severe lesions than nonvaccinated animals after *M. bovis* infection, as described here and by Nol et al. (14), generally harbor lower and/or diminishing levels of detectable circulating antibodies to *M. bovis* antigens than nonvaccinated deer with more advanced disease. This finding is consistent with those of previous studies, in which associations between the severity of disease caused by *M. bovis* infection and the levels of antibody production were noted (5, 10, 12, 23, 26).

Using RT and/or MAPIA, we were generally able to distinguish vaccinated and *M. bovis*-challenged deer with minimal lesion pathology from nonvaccinated and challenged deer with more advanced lesion development. In the present study, with one exception, none of the vaccinated deer responded by RT or produced detectable antibodies to more than one of the three most useful antigens by MAPIA. One of the two nonvaccinated animals that did not develop a detectable antibody response after infection to at least two of the three antigens analyzed by MAPIA did react by RT postinfection. The single nonvaccinated animal that tested negative by both assays was one of the two animals with the lowest lesion scores. It is possible that, given time, this animal would have reacted by these tests as the disease increased in severity; it is also possible that this animal had some resistance to *M. bovis* infection and may not have experienced a rapid development of pathology.
These tests could serve as antemortem tools to aid researchers with the evaluation of the efficacy of a vaccine against *M. bovis* infection in deer. With this scenario, detection of the production of high levels of antibodies to *M. bovis* antigens would be indicative of vaccine failure.

Antibody-based tests could be used to evaluate the status of B Tb in a free-ranging, vaccinated herd through the testing of live animals. The tests could identify severely affected animals that should be removed from the population to prevent shedding of the organism and the further spread of infection. Vaccine-protected animals with no or minimal lesion development would remain in the herd, as it has been suggested that such vaccinated deer experience minimal pathological changes postinfection and, even though they are infected, are not considered an important source of *M. bovis* to the environment for transmission to other animals (4).

The present study was limited to the evaluation of the humoral immune responses of small numbers of vaccinated and unvaccinated white-tailed deer in the first 4 to 5 months after challenge with *M. bovis*. Studies involving large wildlife species at a high biosafety level often suffer from a low power and a short duration due to the great expense, logistical issues, and animal welfare considerations. Although obstacles exist, it would be extremely useful to conduct follow-up experiments with larger sample sizes and of a longer duration that monitor the postchallenge antibody responses in both vaccinated and unvaccinated deer. BCG-vaccinated white-tailed deer may acquire long-term protection (>1 year) from the development of severe disease, as has been shown in red deer (4). Whether white-tailed deer continue producing similar results by MAPIA, RT, and LAM-ELISA after a year or longer following vaccination and *M. bovis* challenge would be of great interest and utility to a field vaccination program.

The results of immunoblotting with the WCS antigen suggest that the captive deer used in this study had prior exposure to environmental mycobacteria, although we did not isolate these organisms from the deer's tissues. Ongoing studies evaluating the responses of captive and free-ranging deer to various WCS antigens (i.e., from *M. kansasi*, *M. avium* subsp. *paratuberculosis*, and *M. bovis*) indicate that deer are constantly exposed to antigens, presumably ubiquitous mycobacteria present in their environment, that elicit antibody reactive to mycobacterial WCS (6; W. R. Waters, personal observation). Obviously, preexisting responses complicate the analysis of specific responses to vaccination and infection and likely affect vaccine efficacy and disease progression in both experimental and field situations (2, 22).

Proteins at the 32-kDa and the 68- to 70-kDa levels elicited relatively unique antibody response patterns among the treatment groups, as detected by immunoblotting. The 32-kDa protein most likely corresponds to the immunodominant antigen 85 (Ag85) complex. The Ag85 complex is a member of the mycolyltransferase family found in all mycobacteria and represents a major fraction of the proteins secreted in culture filtrates of *M. bovis*, including BCG (17, 27). The Ag85 complex has effectively been used in the form of a DNA vaccine to boost the protective effects of BCG vaccination in mice and cattle. Although it is unclear why the nonvaccinated animals had prolonged responses to the Ag85 complex relative to the lengths of the responses of the other groups, particularly the orally vaccinated groups, it is possible that mucosal exposure to this antigen prior to challenge leads to a reduction in the antibody response over time after challenge and may correspond to a lack of lesion development. Alternatively, effective mucosal vaccination may limit the antigen load detected by the host in the form of antibody production. That the animals in the parenteral vaccination group did not respond in this manner could be a result of the more effective sensitization to the Ag85 complex afforded by vaccination by the parenteral route compared to that afforded by vaccination by the oral routes. The protein(s) in the 68- to 70-kDa range probably corresponds to a heat shock protein (HSP70) that has been shown to be immunogenic (8, 9). It is unclear why the animals in the parenteral and oral bait vaccination groups responded more consistently to this antigen than the animals in the other groups. More research needs to be conducted in order to understand the significance of the responses to both sets of proteins by BCG-vaccinated white-tailed deer.

The animals in this study did not produce readily detectable antibody responses to MPB83 by immunoblotting or MAPIA. This is in contrast to the observations from previous studies with cervids in which MPB83 was found to be immunodominant in these tests (6, 13, 23). However, when this antigen was combined with the 16-kDa protein (also known as Acr1), it was the most readily detected antigen by MAPIA in this study and was one of the most readily detected antigens in other studies as well (6, 13, 23, 24). These results highlight the variability of the responses by deer, as well as the variability of the responses by many other host species, to mycobacterial antigens on both an individual and a herd basis. These types of findings are not limited to *M. bovis* infections and are observed in the face of a variety of mycobacterial infections. This could be due to genetic differences among individuals and herds, as well as environmental factors. The results of this study and others highlight the importance of the use of a multiantigen approach, and a continued search for effective antigens is needed to develop more sensitive and specific serologic assays for the evaluation of vaccines and disease progression, as well as the general diagnosis of *M. bovis* infection in all species. (6, 13, 21, 23).

In conclusion, white-tailed deer that were protected from severe disease due to vaccination with BCG, either orally or parenterally, produced either no detectable antibody responses or decreasing antibody levels, as determined by MAPIA, RT, and LAM-ELISA, compared to the antibody responses and the antibody levels of nonvaccinated deer inoculated with *M. bovis*. These results support the potential of antibody-based assays as useful indicators of vaccine efficacy in experimental vaccine trials as well as for the monitoring of disease in vaccinated free-ranging deer populations. In addition, although the immunoblotting data provided limited insight regarding vaccine-induced protection against disease, they do encourage the further investigation of the immune responses to the Ag85 complex and HSP70 in deer vaccinated with BCG or similarly attenuated *M. bovis* constructs. More research on the development of serologic assays for the detection of immune responses to vaccination and *M. bovis* infection is needed. In addition, more expansive, longer-term studies on the safety and efficacy of BCG against *M. bovis* infection in white-tailed deer are imperative, so that vaccination may ultimately be used for the management of wild deer populations affected by B TB.
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