Mycobacterium avium subsp. paratuberculosis Antibody Response, Fecal Shedding, and Antibody Cross-Reactivity to Mycobacterium bovis in M. avium subsp. paratuberculosis-Infected Cattle Herds Vaccinated against Johne’s Disease

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Vaccination for Johne’s disease with killed inactivated vaccine in cattle herds has shown variable success. The vaccine delays the onset of disease but does not afford complete protection. Johne’s disease vaccination has also been reported to interfere with measurements of cell-mediated immune responses for the detection of bovine tuberculosis. Temporal antibody responses and fecal shedding of Mycobacterium avium subsp. paratuberculosis, the causative agent of Johne’s disease, were measured in 2 dairy cattle herds using Johne’s disease vaccine (Mycopar) over a period of 7 years. Vaccination against Johne’s disease resulted in positive serum M. avium subsp. paratuberculosis antibody responses in both herds, and the responses persisted in vaccinated cattle up to 7 years of age. Some vaccinated animals (29.4% in herd A and 36.2% in herd B) showed no serological reactivity to M. avium subsp. paratuberculosis. M. avium subsp. paratuberculosis-specific antibody responses were also detected in milk from Johne’s disease-vaccinated animals, but fewer animals (39.3% in herd A and 49.4% in herd B) had positive results with milk than with serum samples. With vaccination against M. avium subsp. paratuberculosis, fecal shedding in both dairy herds was reduced significantly (P < 0.001). In addition, when selected Johne’s disease-vaccinated and -infected animals were investigated for serological cross-reactivity to Mycobacterium bovis, no cross-reactivity was observed.

Johne’s disease in cattle is a chronic disease caused by Mycobacterium avium subsp. paratuberculosis. In the United States, the disease causes estimated losses of $200 million every year (1). Control of Johne’s disease is achieved by testing, culling, and improving biosecurity and herd management (2). Vaccination, using killed inactivated vaccines, has also been attempted for disease control. The vaccines are said to afford protection by delaying the onset of clinical disease, but the protection against infection in cattle is not complete (3, 4). Vaccination with a callowhood vaccine is prescribed for replacement heifers and male calves. Currently available vaccines have the major disadvantages that they cause granulomas, can result in accidental vaccination of humans, and are said to interfere with the bovine tuberculosis (TB) skin test (4). Furthermore, the true cost benefits of vaccinations are unknown, although vaccinations historically have been shown to have economic value (1, 5).

Information regarding the efficiency of Johne’s disease vaccination in cattle herds is scarce, and cross-reactivity in bovine TB tests has been shown to be a problem in vaccinated cattle, small ruminant, and cervid herds (6–12). Additional testing using the comparative cervical test (CCT) or gamma interferon (IFN-γ) measurement helps to determine whether reactivity seen with skin-based screening is specific, but the follow-up testing is often laborious and time-consuming (4, 6).

To study the effects of vaccination in cattle, we selected two dairy herds receiving Johne’s disease vaccination in the wake of natural disease, and we studied M. avium subsp. paratuberculosis-specific antibody responses and fecal shedding of M. avium subsp. paratuberculosis in these two herds. Vaccination or infection with M. avium subsp. paratuberculosis has been shown to result in interference in cell-based bovine TB assays (6–12). With the recent availability of new serological assays to detect bovine TB, the cross-reactivity of M. avium subsp. paratuberculosis-specific antibodies in response to Johne’s disease infection or vaccination was investigated using the IDEXX Mycobacterium bovis antibody enzyme-linked immunosorbent assay (ELISA), which is based on two antigens (MPB83 and MPB70) (13).

MATERIALS AND METHODS

Sample populations. Two herds (designated A and B) that were receiving vaccinations and were part of the Pennsylvania Johne’s Disease Demonstration Herd Project were selected and were studied for 7 years (2004 to 2010).

Sampling plan. Paired blood and fecal samples were collected from all ≥24-month-old animals, both lactating and nonlactating, in both herds, in order to evaluate the M. avium subsp. paratuberculosis antibody responses and shedding status of the individual animals. The two types of samples were collected from each animal in the herd on the same day, identified using unique nontransferable identification numbers, and sent on ice to the laboratory in Harrisburg, Pennsylvania, for processing. Sampling was carried out annually for 7 years, 11 to 13 months after the
previous sampling. A total of 952 animal samplings were carried out, 594 from vaccinated animals and 358 from unvaccinated animals.

In select years, Dairy Herd Improvement Association (DHIA)-collected milk samples were also obtained from all lactating cows. These samples were obtained from the DHIA test date closest to the time of the fecal and blood sampling in selected years. Since both herds were on a monthly sampling schedule, the milk samples were always obtained within 2 weeks of the blood and fecal samples but not on the same day. The milk samples were tested to evaluate the levels of *M. avium* subsp. *paratuberculosis* antibody responses.

Initial and yearly assessments were conducted for both herds, and management changes were recorded. Both farms were tested annually beginning in 2004 (year 1) and continuing through 2010 (year 7). Vaccination was included as part of disease control measures on both farms.

**Vaccination and management changes.** According to state regulations, caudal fold tuberculin testing was performed on all calves prior to the initiation of vaccination. All test results were negative. Calves were vaccinated by the herd veterinarians with 0.5 ml of killed whole-cell vaccine (Mycopar), administered subcutaneously in the brisket area, before 35 days of age. Herd A began receiving vaccination against Johne’s disease prior to the initiation of this study; therefore, by year 1 of the study, there were 116 vaccinated animals >24 months of age in the herd. The proportions of vaccinated animals (versus unvaccinated animals) sampled in this herd ranged from 63.7% (74/116 animals) in 2004 to 100% (122/122 animals) in 2007. Herd B initiated *M. avium* subsp. *paratuberculosis* vaccination late in 2002 and did not have any vaccinated animals >24 months of age by the time of the first sampling in 2004. In year 7, 98% of the animals (49/50 animals) that underwent sampling had received the calfhood vaccination.

In addition to vaccination, herd A implemented numerous changes designed to minimize the probability of new infections, based on a Johne’s disease risk assessment (http://www.johnesdisease.org/RiskAssessment%20ManagementPlans%20for%20Johne%27s.pdf). The most significant changes included improved hygiene of the maternity area, prompt removal of the calves from the maternity area, and development of a protocol to castrate any test-positive cows in a separate location. In addition, sick animals were not housed in the maternity area, and coostrum from any test-positive or untested animals was not used for heifer calves. Almost all fecal culture-positive animals were culled from the herd shortly after diagnosis, although animals with very low levels of shedding occasionally were retained for longer periods. Special attention was paid to these animals, to monitor them for clinical signs, and precautions (see above) were taken to minimize the risk of new infections resulting from these animals.

Numerous risk areas were identified in herd B by means of the risk assessment. However, this farm elected to make very few substantive changes in their management practices, although positive animals were identified. Some attempts were made to not have heavy shedders or clinically ill animals calve in the maternity area. Sick animals were generally not housed in the maternity area, although this did occur. For financial reasons, herd B retained many of its serum- and fecal-culture-positive animals, although there were concurrent reasons to remove the animals (e.g., high somatic cell counts or other disease concerns) or they began showing clinical signs. Most but not all replacements for herds A and B were home-raised.

**ELISAs.** The IDEXX *Mycobacterium paratuberculosis* ELISA kit (HerdChek *M. pt.* kit) for detection of *M. avium* subsp. *paratuberculosis*-specific antibodies in serum and the IDEXX *Mycobacterium bovis* ELISA kit for *M. bovis* (IDEXX, Westbrook, ME) were used in this study. ELISAs were conducted following the manufacturer’s directions, with a 1:20 dilution of 100 µl of serum for the IDEXX HerdChek *M. pt.* kit and a 1:50 dilution of 100 µl of serum for the *M. bovis* ELISA kits. Antibody responses against *M. avium* subsp. *paratuberculosis* in milk and serum samples from vaccinated cattle were compared. Milk samples (100 µl at a 1:20 dilution) from selected animals from both herds (440 samples from herd A and 190 samples from herd B) were tested using the Prionics Paracheck ELISA (Prionics, Switzerland). This kit is approved for testing both serum and milk samples. Positive and negative results were determined according to the kit instructions. Quantitative data were expressed as sample/positive (S/P) ratios for the IDEXX assays or sample/negative values for the Prionics assay (Prionics, Switzerland).

**Agar slant cultures.** Two grams of feces was decontaminated by the double incubation-centrifugation method, as described previously (14). This material was used to inoculate 4 slants of Herrold’s egg yolk medium containing 2 mg/liter mycobactin J (Becton, Dickinson & Co., MD). *M. avium* subsp. *paratuberculosis* colonies were confirmed by using acid-fast staining and an IS900 PCR assay.

**Statistical analyses.** Proportions of vaccinated and unvaccinated animals were calculated for each category in different study years. The effects of vaccination on serological responses and *M. avium* subsp. *paratuberculosis* shedding were analyzed with chi-square analysis. Relative risks from *M. avium* subsp. *paratuberculosis* exposure of vaccinated and unvaccinated groups were calculated. Correlation coefficients (r) were calculated for correlations between the serum and milk ELISA results, to understand the relationship. Serum S/P ratios for selected animals (n = 14) were plotted as scatter or line plots, to show antibody responses following vaccination.

**RESULTS**

**Antibody responses and fecal shedding.** Chi-square statistical analysis showed that vaccination was strongly associated with positive serum responses and reduction of fecal shedding of *M. avium* subsp. *paratuberculosis* in both herds (*P* < 0.001). Unvaccinated cattle in herd A showing no evidence of infection were predominately seronegative for *M. avium* subsp. *paratuberculosis* antibodies, as indicated by comparing positive ELISA results for the total and vaccinated animal categories (Table 1). Significant proportions of vaccinated animals showed antibody responses (ranging from 32.7% to 70.9% in different years) and were found to be fecal culture negative for *M. avium* subsp. *paratuberculosis* (*P* < 0.001). The rate of seropositivity was highest in 2006, when >70.4% of animals tested positive (Table 1). A substantial number of vaccinated animals remained serologically negative (29.4% in herd A). At the start of the study, the within-herd prevalence rate was 6%. In the vaccinated group, fewer animals shed *M. avium* subsp. *paratuberculosis* in feces, and those that were ELISA positive shed fewer organisms (maximum, 3.2%). The whole-herd fecal culture-positive rate was also maintained at a low level. Some animals were fecal culture positive but remained seronegative in response to both *M. avium* subsp. *paratuberculosis* infection and vaccination. Table 1 shows the total fecal culture-positive and ELISA-positive results in response to either vaccination or infection. For some animals that showed seropositive responses, antibody levels did drop below the cutoff value of 0.25 or to the negative range, as determined by S/P ratios (Fig. 1A). However, for many other animals that could be traced for several years in both herds A and B, animals remained positive for serum *M. avium* subsp. *paratuberculosis* antibodies in the fifth year of the study or 7 years following vaccination (Fig. 1B).

The second herd, herd B, initially showed considerable rates of infection (>20% within-herd prevalence) in both the vaccinated and unvaccinated groups. The fecal shedding rate was 23.2% in 2004, at the start of the study, and remained above 20% until 2006, with several high-level shedders being identified within the herd (Table 1). ELISA positivity rates ranged between 51 and 100% among vaccinated animals in any given study year. Overall, 36.2% of vaccinated animals showed no ELISA reactivity. Fecal shedding dropped from an initial rate of greater than 20% to less than 10%
TABLE 1  *Mycobacterium avium* subsp. *paratuberculosis* annual antibody responses and fecal shedding in *M. avium* subsp. *paratuberculosis*-infected cattle herds receiving *M. avium* subsp. *paratuberculosis* vaccination with management changes (herd A) or without management changes (herd B)

<table>
<thead>
<tr>
<th>Herd and yr</th>
<th>No. in herd*</th>
<th>Vaccinated</th>
<th>Total</th>
<th>Vaccinated</th>
<th>ELISA positive</th>
<th>Fecal culture positive</th>
<th>ELISA positive</th>
<th>Fecal culture positive</th>
<th>ELISA positive</th>
<th>Fecal culture positive</th>
<th>Vaccinated fecal culture positive</th>
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<td>A</td>
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<tr>
<td>2004</td>
<td>116</td>
<td>74 (63.7)</td>
<td>49 (42.2)</td>
<td>7 (6)</td>
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<td>2005</td>
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<td>105 (90.5)</td>
<td>74 (63.7)</td>
<td>1 (0.8)</td>
<td>73 (69.5)</td>
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<td>2006</td>
<td>115</td>
<td>110 (95.6)</td>
<td>78 (67.8)</td>
<td>3 (2.4)</td>
<td>78 (70.9)</td>
<td>1 (0.9)</td>
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<tr>
<td>2007</td>
<td>122</td>
<td>122 (100)</td>
<td>40 (32.7)</td>
<td>2'/3 (2.4)</td>
<td>40 (32.7)</td>
<td>2'/3 (2.4)</td>
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<tr>
<td>2008</td>
<td>125</td>
<td>125 (100)</td>
<td>65 (52.0)</td>
<td>3'/4 (3.2)</td>
<td>65 (52)</td>
<td>3'/4 (3.2)</td>
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<td>2009</td>
<td>128</td>
<td>94 (73.4)</td>
<td>49 (38.2)</td>
<td>2'/3 (2.3)</td>
<td>49 (52.2)</td>
<td>1 (1)</td>
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<tr>
<td>2010</td>
<td>128</td>
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<td>35 (27.3)</td>
<td>1 (0.7)</td>
<td>34 (51.5)</td>
<td>0</td>
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<td>2004</td>
<td>43</td>
<td>0</td>
<td>3 (6.6)</td>
<td>10 (23.2)</td>
<td>2 (100)</td>
<td>1 (50)</td>
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<tr>
<td>2005</td>
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<td>2 (4.4)</td>
<td>7 (15.5)</td>
<td>8'/11 (24.4)</td>
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<tr>
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<td>30 (57.6)</td>
<td>8'/11 (21.1)</td>
<td>26 (53.0)</td>
<td>2'/3 (6.1)</td>
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<tr>
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<td>27 (44.2)</td>
<td>3'/4 (7.2)</td>
<td>32 (61.5)</td>
<td>3'/4 (7.6)</td>
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<td>2008</td>
<td>55</td>
<td>52 (94.5)</td>
<td>33 (60)</td>
<td>3'/5 (9.0)</td>
<td>31 (60.7)</td>
<td>5'/6 (11.7)</td>
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<td>2009</td>
<td>52</td>
<td>51 (98)</td>
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*The numbers of animals >24 months of age are indicated for each study year.

*Total and vaccinated ELISA- and *M. avium* subsp. *paratuberculosis* fecal culture-positive animals are indicated as ELISA positive and fecal culture positive, respectively. Animals that were ELISA positive and were *M. avium* subsp. *paratuberculosis* fecal culture positive in the total and vaccinated groups are indicated as fecal culture positive and vaccinated fecal culture positive, respectively, under ELISA positive.

*New infections for each year are indicated, showing totals for new infections and total positive results.

in subsequent years. Vaccinated animals were found not to be fully protected in herd B, as was the case in herd A. Several animals (range, 4.0 to 50%) were found to be shedding *M. avium* subsp. *paratuberculosis* and were identified as high shedders even after being vaccinated. Overall, the numbers of *M. avium* subsp. *Paratuberculosis*-shedding animals decreased with time. In herd B, similar to herd A, some animals were fecal culture positive for *M. avium* subsp. *paratuberculosis* but were serologically negative in both the vaccinated and infected groups.

**Serum and milk antibody responses.** Positive correlations between the serum and milk antibody responses were noticed for both vaccinated herds (herd A, r = 0.69; herd B, r = 0.63). Many vaccinated animals showed measurable *M. avium* subsp. *Paratuberculosis*-specific antibody responses in serum but showed no specific antibodies for *M. avium* subsp. *paratuberculosis* in milk. In herd A, 440 sample pairs collected from lactating vaccinated animals were tested with milk and serum ELISAs; 173 (39.3%) were found to be positive for *M. avium* subsp. *paratuberculosis*-specific antibody responses by serum testing but were negative by milk testing. In herd B, among the 141 vaccinated animals sampled, 51 (49.0%) tested positive in serum testing but negative when milk was analyzed for *M. avium* subsp. *paratuberculosis*-specific responses. There was no major difference in serum and milk antibody responses among the unvaccinated animals (n = 104), including the animals that were shedding *M. avium* subsp. *paratuberculosis*. For 5/104 animals (4.8%) in herd A and 7/125 animals (5.6%) in herd B, although the animals were shedding *M. avium* subsp. *paratuberculosis*, both milk and serum ELISAs failed to detect infected animals among the unvaccinated animals. In the same unvaccinated category, 93% of animals sampled for all 3 tests (fecal, serum, and milk testing) were negative.
Serological cross-reactivity to *Mycobacterium bovis*. Sera that were strongly positive for *M. avium* subsp. *paratuberculosis* antibody responses (S/P ratios) due to infection or vaccination were tested with a *M. bovis* ELISA. Our results showed no reactivity with the *M. bovis* ELISA, although the animal sera were positive for *M. avium* subsp. *paratuberculosis* antibodies. Twenty serum samples with S/P ratios of >0.25, 10 serum samples with S/P ratios of <0.25 from animals vaccinated against *M. avium* subsp. *paratuberculosis*, and 16 serum samples with S/P ratios of >0.25 from *M. avium* subsp. *paratuberculosis*-infected animals that were serologically positive were included in this comparison. None of the sera showed any reactivity to *M. bovis*.

**DISCUSSION**

Vaccination of calves with a killed vaccine has not been shown to completely prevent *Mycobacterium avium* subsp. *paratuberculosis* infection but is considered to be an effective tool for controlling the spread of disease (8). Another study showed reductions in infection but is considered to be an effective tool for controlling completely prevent *M. avium* subsp. *paratuberculosis* infection. Twenty serum samples with S/P ratios of >0.25, 10 serum samples with S/P ratios of <0.25 from animals vaccinated against *M. avium* subsp. *paratuberculosis*, and 16 serum samples with S/P ratios of >0.25 from *M. avium* subsp. *paratuberculosis*-infected animals that were serologically positive were included in this comparison. None of the sera showed any reactivity to *M. bovis*.

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TB surveillance. Cattle and deer vaccinated with killed *M. avium* subsp. *paratuberculosis* vaccine have been reported to demonstrate antibody responses to *M. avium* subsp. *paratuberculosis* and also to *M. bovis* (6, 12). Using the new IDEXX *M. bovis* assay (using MPB70 and 83 antigens), we did not see any cross-reactive antibody responses against bovine TB in vaccinated cattle in *M. avium* subsp. *paratuberculosis*-vaccinated or -infected animals. Our findings are supported by another study, in which testing of deer herds to decrease infection levels on farms.

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... only testing-negative animals enter the herd are some approaches to decrease infection levels on farms. Vaccines interfering with TB testing, but this needs to be

Detection of antibodies to these antigens is boosted by skin tuberculin testing when cattle are infected with bovine TB (25). Although animals were not tested in our study soon after tuberculin skin testing, animals that received both tuberculin testing and vaccination against Johne’s disease did not show any seroreactivity to *Mycobacterium tuberculosis* despite showing positive responses to *M. avium* subsp. *paratuberculosis*. In a previous study in which vaccination with *M. avium* subsp. *paratuberculosis* was followed by tuberculin skin testing, the specificity of *M. tuberculosis* antigens in serological assays was not compromised by *M. avium* subsp. *paratuberculosis* vaccination (7).

Vaccination can be a useful strategy for the management of Johne’s disease but is not used frequently due to the associated risks (4). Concerns about *M. avium* subsp. *paratuberculosis* vaccination interfering with the interpretation of diagnostic tests for *M. avium* subsp. *paratuberculosis* or bovine TB are valid. Current *M. avium* subsp. *paratuberculosis* vaccines do not afford full protection but do offer economic promise while better vaccines are being researched (26, 27). The newer serological bovine ELISAs may help address some of the concerns about *M. avium* subsp. *paratuberculosis* vaccines interfering with TB testing, but this needs to be further evaluated in geographically diverse settings. Until vaccines that afford better protection are developed, using good management practices on farms and reducing the numbers of high-level shedders, controlling vertical transmission, and making sure that only testing-negative animals enter the herd are some approaches to decrease infection levels on farms.

**REFERENCES**


