

Evaluation of the effects of a killed whole-cell vaccine against *Mycobacterium avium* subsp *paratuberculosis* in 3 herds of dairy cattle with natural exposure to the organism

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Objective—To evaluate effects of vaccination with a killed whole-cell vaccine against *Mycobacterium avium* subsp *paratuberculosis* (MAP) on fecal shedding of the organism, development of clinical paratuberculosis (Johne's disease [JD]), milk production, measures of reproduction, and within-herd longevity of dairy cattle naturally exposed to MAP.

Design—Controlled clinical trial.

Animals—200 vaccinated and 195 unvaccinated (control) dairy cows from 3 herds in Wisconsin.

Procedures—Every other heifer calf born in each herd received the MAP vaccine; 162 vaccinates and 145 controls that had ≥ 1 lactation were included in analyses. Bacteriologic culture of fecal samples for MAP was performed annually for 7 years; results were confirmed via histologic methods and PCR assay. Production records and culture results were evaluated to determine effects of vaccination on variables of interest in study cows. Annual whole-herd prevalence of MAP shedding in feces was also determined.

Results—Vaccinates had a significantly lower hazard of testing positive for MAP via culture of fecal samples than did controls over time (hazard ratio, 0.57; 95% confidence interval, 0.34 to 0.97). Fewer vaccinates developed clinical JD than did controls ($n = 6$ and 12 , respectively), but these differences were nonsignificant. Overall within-herd longevity, total milk production, and calving-to-conception intervals were similar between vaccinates and controls. In all herds, prevalence of MAP shedding in feces decreased over time.

Conclusions and Clinical Relevance—Vaccination with a killed whole-cell MAP vaccine appeared to be an effective tool as part of a program to control the spread of JD in dairy cattle. (*J Am Vet Med Assoc* 2013;242:663–669)

Paratuberculosis (JD) is a chronic proliferative gastroenteritis of cattle, sheep, and goats caused by the acid-fast organism MAP.¹ There is no economically feasible cure for the disease, and animals with clinical signs are generally culled from the herd. This can result in large economic losses, particularly for dairy cattle producers. Indirect losses attributable to JD include reduced milk production, longer calving-to-conception interval, and increased risk of culling.^{2,3} The epidemiology of JD is further complicated by the fact that infected animals can shed the organism into the environment without having clinical signs. The causative bacteria can remain viable in the environment for weeks to months, depending on environmental conditions, facilitating indirect transmission from infected to

| ABBREVIATIONS | |
|---------------|--|
| CI | Confidence interval |
| IS900 | Insertion sequence 900 |
| JD | Johne's disease |
| MAP | <i>Mycobacterium avium</i> subsp <i>paratuberculosis</i> |

susceptible animals. Detection of low-level or intermittent shedders is challenging because diagnostic tests have low sensitivity to detect animals prior to late stages of disease.⁴

Numerous programs have been developed to control JD in dairy cattle, with a goal to reduce disease transmission within herds.⁵ Environmental control measures

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primarily focus on herd management techniques to reduce effective contacts between mature cattle shedding MAP and susceptible young stock.⁶ Testing and culling of test-positive adult cattle is also practiced, and most JD control programs use a combination of management and testing programs.² This strategy has been shown to reduce the proportion of cows with subclinical MAP infection and clinical JD in dairy herds.⁷ However, successful disease control requires years of effective management change, and in some herds for which owners are unwilling or unable to implement effective changes, clinical cases of JD continue to occur years and even decades after a control strategy has been enacted.

Vaccination against MAP has been practiced in a controlled and limited manner since the 1920s.⁸ The vaccine currently available in the United States is a killed whole-cell bacterin in oil adjuvant.^a A single dose is administered SC in the brisket area to dairy calves within 35 days after birth. Cattle administered the vaccine have an increased likelihood of a positive caudal fold tuberculin test response and subsequent comparative cervical tuberculin test response regardless of actual *Mycobacterium bovis* infection status,⁹ acting as a confounder in detecting tuberculosis in cattle. In the 17 states that currently allow use of the MAP vaccine in cattle,^b tuberculin testing of the entire herd is performed prior to vaccination. This is done to establish a baseline of no *M bovis* infection in the herd before exposing the immune system to MAP antigen. All vaccinated cattle are uniquely identified with an official ear tag. Following vaccination, many cattle develop a permanent granuloma at the injection site, although this has not been found to cause long-lasting effects.¹⁰ Use of the vaccine is regulated by the USDA because of these described effects.¹¹

Several studies have been performed to evaluate the effects of vaccination against MAP in cattle and sheep,¹ and vaccination has been found to reduce the incidence of clinical JD,^{10,12,13} with partial reduction in fecal shedding of MAP.^{14,15} A recent meta-analysis^c resulted in similar conclusions. Findings in these studies have been limited due to lack of a comparison group in the study design or insufficient duration to allow evaluation of long-term impacts. In many studies, results from a single bacteriologic culture of fecal samples from vaccinates were either compared with culture results for nonvaccinated animals from another farm^{15,16} or with herd prevalence of MAP infection before vaccination was implemented.^{12,13,17} Use of other herds as comparison groups introduces potential bias because the confounding effects of herd management between treatment groups cannot be entirely controlled without randomization, and comparisons of prevalence within a herd before and after implementation of a vaccination program ignore the confounding effect of time.

The chief potential benefits of vaccinating a herd against MAP are reducing the number of animals shedding the organism and reducing the amount of MAP shed per animal. Additional potential benefits include reducing the incidence of clinical JD, extending within-herd longevity in vaccinated animals, improving milk production, and decreasing the calving-to-conception interval. To date, no studies have clearly demonstrated the effects of vaccination against MAP in cattle in

all of these areas. The objective of the study reported here was to evaluate the effects of vaccination with a killed whole-cell vaccine against MAP on fecal shedding of the organism, development of clinical JD, milk production, reproduction, and within-herd longevity in naturally infected dairy herds in a controlled clinical trial. By comparing a cohort of vaccinated and unvaccinated (control) calves reared under the same management conditions, we sought to evaluate vaccination as a management tool in JD control programs.

Materials and Methods

Animals and study design—Three dairy herds in western Wisconsin were selected for study participation on the basis of adult cow herd size (≥ 300 milking cattle/herd), infection prevalence ($\geq 10\%$ apparent seroprevalence via ELISA), maintenance of electronic records, and owners' willingness to participate in the project. The study took place on private unaffiliated dairy farms, and institutional approval was not required. Prior to enrollment, all 3 herds were assessed as being infected with MAP via a combination of serum ELISA, bacteriologic culture of fecal samples, and reported clinical cases of JD. All farms had participated in MAP testing and management programs for 3 to 5 years prior to the start of the study and maintained computerized records^d documenting infection prevalence, cull rates, and productivity prior to initiating the vaccination program. All herds had risk assessments for MAP infection performed annually, and individualized herd management plans were developed in line with the owners' goals for each herd. Management changes were documented, and vaccination was incorporated in combination with other disease control measures. Caudal fold tuberculin testing of all cattle was performed in each herd prior to starting the vaccination program to ensure animals were free from *M bovis* infection.

Herds in the study ranged in size from 325 to 825 cows, with a combined total of approximately 1,800 lactating cows. Prior to participating in the study, annual culling losses attributable to clinical JD accounted for approximately 6% to 8% of the milking herds. In each herd, free-stall housing was used for lactating and dry cows, with separate housing for heifers and calves. Calves were removed ≤ 20 minutes after birth (before suckling) to a separate housing facility and fed colostrum from a dam that tested negative for MAP via ELISA or bacteriologic culture of feces. All heifer calves were subsequently fed milk replacer or pasteurized waste milk (no bull calves were included in the study). Prior to the start of the study, cows in all herds were tested via serum ELISA to detect MAP antibody at the end of lactation. Cows that tested positive via ELISA were kept in maternity pens separate from the rest of the herd, and no colostrum collected from these cows was fed to replacement heifer calves. In 2 of the 3 herds (2 and 3), only heifer calves from ELISA-negative dams were retained for inclusion in the milking herd and the study.

From September 1, 2003, through July 1, 2004, every other heifer calf born in each herd was administered 1 dose of killed whole-cell vaccine against MAP^a (0.5 mL, SC) in the brisket region between 1 and 35 days of age,

according to manufacturer's instructions. The vaccine was administered by one of the study investigators (JJB). Vaccinated cattle were uniquely identified with a JD vaccine ear tag. Within each herd, each experimental group included ≥ 50 calves or a number of calves equivalent to 10% of the adult cow herd, whichever was greater. After the initial vaccinate and control groups were established, all heifer calves subsequently born in the enrolled herds were vaccinated.

Fecal samples from vaccinates and controls were manually collected from the rectum at the time of first calving, at 90 days of gestation in each lactation, and at time of culling (regardless of reason). Samples were stored in individually labeled containers and refrigerated at 5°C until received at the laboratory. Fecal samples collected from all adult cattle in enrolled herds at 90 days of gestation between July 1 and June 30 of the following year were evaluated for the following intervals: 2004 to 2005 (study year 0; the year after initial vaccination and prior to the first study cohort entering the milking herd), 2005 to 2006 (study year 1), 2006 to 2007 (study year 2), 2007 to 2008 (study year 3), 2008 to 2009 (study year 4), and 2009 to 2010 (study year 5).

Fecal samples were tested at the Wisconsin Veterinary Diagnostic Laboratory in Madison, Wis, to detect MAP with a broth-based commercial liquid bacteriologic culture system,^{e,f} as has been described.¹⁸ Culture medium of samples that had a positive result or of samples that had a negative result after 5 weeks of culture was evaluated to confirm findings via standard acid-fast staining and PCR testing to detect IS900 DNA. A commercially available kit^g was used for the IS900 DNA extractions according to manufacturer's instructions, and a conventional PCR assay was performed followed by gel electrophoresis.¹⁹ Samples were scored as positive if growth was detected via the liquid culture system within 5 weeks or if organisms compatible with MAP were detected with acid-fast staining and results of the PCR assay were positive for IS900 DNA in the fecal sample.

Statistical analysis—Production records were collected biannually, and current lactation and culling information was obtained. Culling date and reason were recorded for all cows in the vaccinate and control groups. Results of annual fecal MAP culture were collected directly from the testing laboratory. The whole adult-herd prevalence of MAP shedding was estimated annually, and a Cochran-Armitage trend test was used to compare the prevalence of positive results for culture of feces among study years, stratified by herd.²⁰

For the control and vaccinate groups, incidence rate for detection of MAP in fecal samples was calculated by dividing the number of events of interest by the total follow-up time for cows at risk of having the event for the first time. Cows were classified as having a positive culture result if they ever tested positive for MAP via bacteriologic culture of feces, and culled cows for which clinical JD given as the reason for culling were classified as having clinical JD. Univariate comparisons between vaccinates and controls were performed via Mantel-Haenszel χ^2 test statistics and relative risk calculation.

Survival analysis was used to compare the hazards of fecal shedding of MAP, culling due to clinical JD, and

removal from the herd for any reason between vaccinates and controls. Cows that were culled or died prior to their first lactation were not included in the survival analysis because no culture results were available and these cows did not survive long enough to develop clinical JD. The time to a positive culture result was calculated by subtracting the date of first positive culture result from the date of birth for all study cattle that survived to first lactation. If a cow never tested positive, it was censored on the last date tested.

Within-herd longevity was calculated by subtracting the date of birth from the date of removal, including cows that died. Cows remaining in the herd were censored with the last date of record on the farm. Time to culling for cows with clinical JD was calculated similarly. Time to first positive culture result, time to culling for cows with clinical JD, and overall survival time were compared between vaccinates and controls via Kaplan-Meier survival curves. Three Cox proportional hazards regression models were built to evaluate the outcomes of time to first positive culture result, time to culling because of clinical JD, and time to culling for any reason with vaccination status as the explanatory variable. The effect of herd was controlled via stratification.

Total milk production by lactation was analyzed via a mixed model with a maximum of 5 measurements/cow (lactations 1 to 5).²¹ The correlation of total milk production between lactations within cows was modeled with a Toeplitz covariance structure, which was chosen on the basis of the Akaike information criterion. Other covariance structures tested included compound symmetry, unstructured, and first-order autoregressive. Herd was included in the model as a random effect, and days in milk, lactation, negative or positive results of MAP culture, treatment group (vaccinated vs control), and the 2-way interactions of experimental group with lactation and experimental group with culture result were included as fixed effects. With the exception of experimental group, variables were included in the final model if $P < 0.10$. Culture result was treated as a time-dependent variable, and if a cow had a positive culture result, its status remained positive for the remainder of the follow-up period.

Date of conception was designated as the last breeding date before the cow was confirmed pregnant by the herd veterinarian. The calving-to-conception interval was calculated by subtracting the date of calving from the date of conception in that same lactation and was analyzed via a proportional hazards regression, with a recurrent event Cox regression approach to model the calving-to-conception interval for each cow across lactations, comparing vaccinates and controls. The conditional gap-times method was used, where time was set to 0 at the beginning of each period, and the events in subsequent lactations were not influenced by the time to event of preceding lactations.²² The variables of herd and culture result were included, and the model was stratified by lactation. Standard errors were adjusted for correlation of observations within the same cow with a robust sandwich variance estimator.

The outcome of pregnancy following first breeding was analyzed via relative risk regression adjusted for group, herd, and culture result. This method was

performed by fitting a binomial distribution with a log link function to estimate relative risks. Relative risks were calculated rather than ORs because in a common event or outcome such as pregnancy (> 10%), the OR will overestimate the magnitude of the effect.

Data were analyzed with standard statistical software.^h Values of $P < 0.05$ were considered significant.

Results

Three hundred ninety-five calves were enrolled in the study, comprising a cohort of 200 calves vaccinated against MAP and 195 unvaccinated controls. Of these, 307 cows for which survival data were available (162 vaccinates and 145 controls) had ≥ 1 lactation and were included in subsequent analyses. The proportion of vaccinates and controls removed from herds prior to first lactation was not significantly ($P = 0.10$) different, nor was the mean age at first calving ($P = 0.90$). Herd distribution of the remaining cows was as follows: herd 1 included 39 vaccinates and 35 controls; herd 2 included 58 vaccinates and 50 controls, and herd 3 included 65 vaccinates and 60 controls. The total number of lactations for vaccinates and controls during the study period was 417 and 370, respectively.

In all 3 herds, a significant (2-sided; $P < 0.001$) trend of decreasing whole-herd prevalence of MAP shedding in feces (determined on the basis of culture results) was detected with a Cochran-Armitage trend test (Table 1). Overall, 23 of 162 (14.2%) vaccinates and 34 of 145 (23.4%) controls had ≥ 1 positive MAP culture result by the end of the study. Significantly ($P = 0.037$) more controls had a positive culture result than did vaccinates. The incidence rate of a positive culture result was 3.11/100 cow-years in vaccinates and 5.22/100 cow-years in controls, with an incidence rate ratio of 0.6. For cattle that had a positive culture result, the mean time to first positive result for vaccinates was 1,370 days, compared with 1,216 days for controls (Figure 1). Proportional hazards regression analysis, with vaccination status as an explanatory variable and stratified by herd, indicated that vaccinates had a significantly ($P = 0.04$) lower hazard of having a positive culture result than did controls over time (hazard ratio, 0.57 [95% CI, 0.34 to 0.97]).

Clinical JD developed in 6 of 162 (3.7%) vaccinates and 12 of 145 (8.3%) controls; this difference was not significant ($P = 0.09$). Incidence rate of clinical JD was 0.78/100 cow-years in vaccinates and 1.74/100 cow-years in controls, with an incidence rate ratio of 0.4. Among cows that developed clinical JD, the mean number of days to development of clinical JD was 1,452 days for controls and 1,911 days for vaccinates (Figure 2). Proportional hazards regression analysis of the herd-stratified data revealed that the risk of culling because of clinical JD over time was not significantly ($P =$

0.067) different for vaccinates, compared with controls (hazard ratio, 0.38 [95% CI, 0.13 to 1.07]).

By the time of final analysis in July 2010, 133 of 162 (82%) vaccinates and 125 of 145 (86.2%) controls had been removed from the herds. Vaccinates remained in the herd a mean of 1,733 days, and controls survived a mean of 1,739 days (Figure 3). The herd-stratified proportional hazard ratio for vaccinated cows being removed from the herd over time, compared with con-

Table 1—Whole-herd prevalence of positive results for MAP culture of fecal samples from lactating cows in a study of 3 dairy herds (herds 1 to 3) in Wisconsin following implementation of programs to control MAP infection, including use of a killed whole-cell MAP vaccine.

| Herd | Study year | | | | | |
|------|------------|------|------|------|------|------|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | 0.29 | 0.21 | 0.16 | 0.12 | 0.14 | 0.06 |
| 2 | 0.16 | 0.13 | 0.09 | 0.12 | 0.05 | 0.03 |
| 3 | 0.13 | 0.08 | 0.05 | 0.06 | 0.07 | 0.05 |

At the start of the study, each herd included ≥ 300 milking cattle and had $\geq 10\%$ apparent seroprevalence of antibodies against MAP. From September 2003 through April 2004, every other heifer calf born in each herd received the MAP vaccine (0.5 mL, SC, once) at 1 to 35 days of age; 162 vaccinates and 145 controls had ≥ 1 lactation. All heifer calves born in the enrolled herds after study groups were established received the vaccine. Similar management programs were implemented in all herds for control of JD. Culture of fecal samples was performed annually at 90 days of gestation for all cows; results were confirmed via histologic methods and PCR assay. A Cochran-Armitage trend test was used to compare the prevalence of positive culture results among study years, stratified by herd. Study years (July 1 to June 30) were defined as follows: 2004 to 2005 (year 0), 2005 to 2006 (year 1), 2006 to 2007 (year 2), 2007 to 2008 (year 3), 2008 to 2009 (year 4), and 2009 to 2010 (year 5).

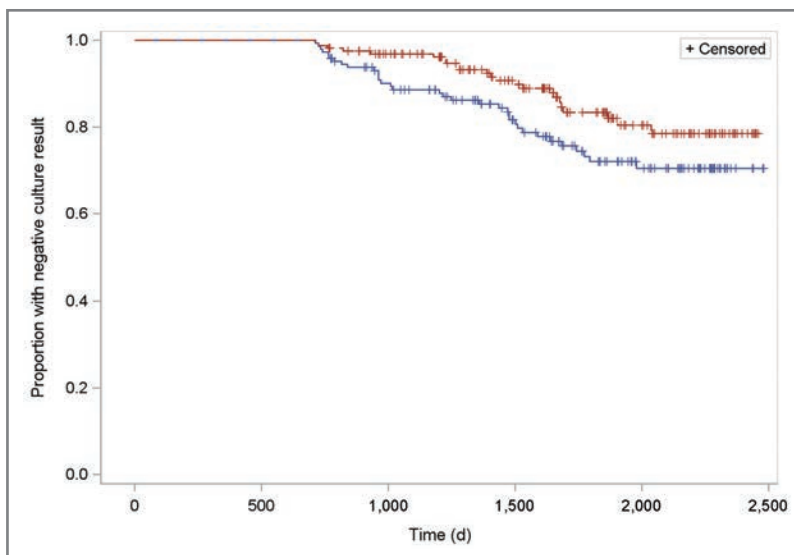


Figure 1—Kaplan-Meier survival curve of the number of days to first positive culture result (ie, the first fecal sample from which MAP was isolated) in cattle that did (vaccinates; red line) or did not (controls; blue line) receive a single dose of killed whole-cell MAP vaccine in a study to evaluate effects of vaccination on various outcomes in 3 herds of dairy cattle. Culture results were confirmed via histologic methods and PCR assay. From September 2003 through April 2004, every other heifer calf born in each herd received the MAP vaccine (0.5 mL, SC, once) at 1 to 35 days of age; 162 vaccinates and 145 controls had ≥ 1 lactation and were included in the analysis. Similar management programs were implemented in all herds for control of JD. Samples for bacteriologic culture were collected from study cows at the time of first calving, in each lactation period at 90 days of gestation, and at the time of culling throughout the 7-year study.

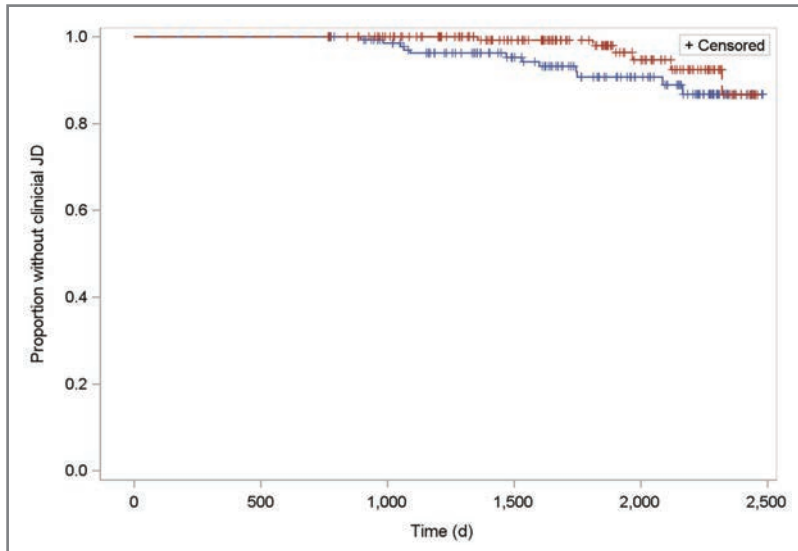


Figure 2—Kaplan-Meier survival curve of the number of days to culling because of clinical JD in the same 307 cattle as in Figure 1. See Figure 1 for key.

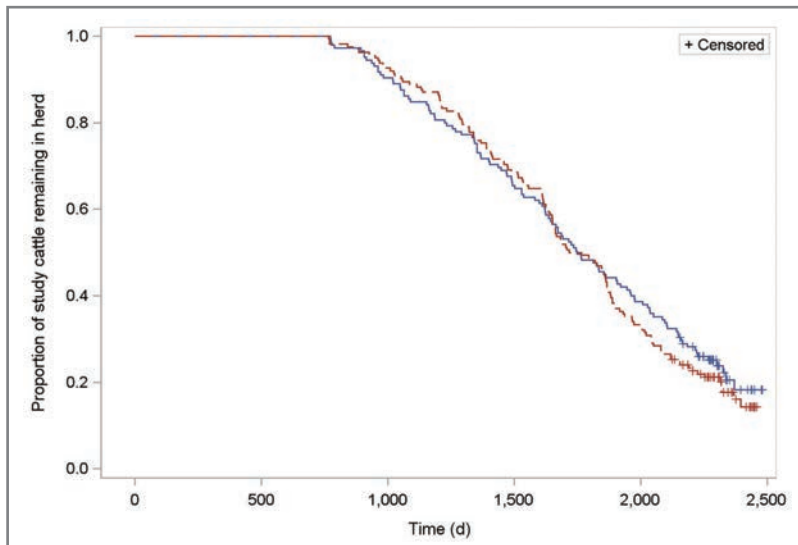


Figure 3—Kaplan-Meier survival curve of the number of days to culling for any reason in the same 307 cattle as in Figure 1. See Figure 1 for key.

trols, was not significantly ($P = 0.65$) different (hazard ratio, 1.06 [95% CI, 0.82 to 1.37]).

There was no significant difference between vaccinates and controls across lactations in the proportion of cows pregnant following first breeding, nor was a significant difference found between controls and vaccinates in the calving-to-conception interval across lactations (Table 2). Second-lactation cows that had negative culture results (regardless of vaccination status) had a significantly shorter calving-to-conception interval, compared with those that had positive culture results (137 vs 156 days; hazard ratio, 0.65 [95% CI, 0.44 to 0.96]).

Vaccination against MAP had no significant ($P = 0.56$) effect on total milk produced across lactations, with herd modeled as a random effect. With herd as a random effect, adjusting for days in milk and lactation, culture results (positive or negative) had no significant ($P = 0.30$) effect on total milk production.

Discussion

In the present study, vaccination against MAP significantly reduced the risk of fecal MAP shedding in dairy cows over time. An important strength of this study was that vaccinates and controls were assigned via systematic selection and raised in the same conditions on each of the 3 study farms, which minimized the effect of confounding by differences in management and JD control efforts. All herds enrolled in this study implemented a JD control program that integrated best management practices with vaccination. Additionally, we monitored the study cattle for 7 years, gaining a long-term perspective on the effect of

Table 2—Summary of fertility measures for study cattle that did (vaccinates) or did not (controls) receive a single dose of killed whole-cell MAP vaccine, stratified by lactation.

| Variable | Lactation | | | |
|---------------------------------------|------------------|-------------------|------------------|------------------|
| | 1 | 2 | 3 | 4 |
| No. of cattle | | | | |
| Vaccinates | 159 | 115 | 66 | 33 |
| Controls | 142 | 101 | 71 | 27 |
| Pregnant after first breeding | | | | |
| Vaccinates (%) | 41.6 | 34.8 | 33.3 | 24.2 |
| Controls (%) | 39.6 | 35.6 | 26.8 | 48.2 |
| Hazard ratio (95% CI)* | 0.95 (0.72–1.26) | 0.98 (0.68–1.40) | 1.25 (0.75–2.08) | 0.50 (0.25–1.03) |
| Calving-to-conception interval | | | | |
| hazard ratio (95% CI)† | | | | |
| Vaccinates vs controls | 1.03 (0.81–1.31) | 0.97 (0.73–1.29) | 1.30 (0.89–1.92) | 1.11 (0.61–2.02) |
| Culture result (negative vs positive) | 0.76 (0.47–1.21) | 0.65 (0.44–0.96)‡ | 1.18 (0.76–1.83) | 0.78 (0.35–1.74) |

*Results of Cox regression analysis of vaccinates versus controls, adjusted for herd and culture result.

†Recurrent Cox model hazard ratios. ‡The calving-to-conception interval was significantly lower for cattle with a negative result for MAP culture of feces, compared with the interval for cattle that had a positive result, regardless of vaccination status.

vaccination over the animals' lifetimes. Previously, few controlled clinical trials had been performed to evaluate the effect of vaccination on JD in dairy cattle. In the 1970s, Larsen et al¹⁰ reported results of a study in which both vaccinates and control cattle were reared on the same farms, but comparisons between groups were limited to postmortem results of bacteriologic culture of feces and incidence of clinical disease at the end of the second lactation. Investigators in that study found a significantly lower proportion of vaccinated animals with clinical JD were culled, although they did not detect a significant difference in the rate of MAP-positive culture results for fecal samples.

Our finding that fewer vaccinated cattle had detectable fecal shedding, compared with controls, provides new evidence for the role of vaccination in reducing herd-level prevalence of MAP infection and in achieving disease control. Effective vaccination of a population has an impact beyond protection of the individuals that directly receive the vaccine.²³ The concept of herd immunity deals with the indirect protection that occurs when vaccinated animals shed fewer infective organisms into the environment or do so for a shorter period of time, thereby providing indirect protection to unvaccinated animals. In effect, the intensity of infectious disease transmission is reduced, which can benefit all animals susceptible to infection.²⁴ Results of the present study show that although vaccination does not completely prevent all animals from shedding MAP, a smaller proportion of vaccinated cattle will shed detectable concentrations of MAP, compared with controls, thereby reducing the number of cattle contaminating the environment.

This development of herd immunity may have impacted our findings with respect to detecting differences between vaccinates and controls. Vaccination led to a reduced proportion of vaccinated cattle shedding MAP in their feces, so unvaccinated cows were exposed to lower environmental bacterial loads within the herds. These effects may have biased study results (including within-herd longevity and other lactation performance measures) toward the null by indirectly benefiting the control group with the intervention of vaccination. Conversely, vaccinated cows were exposed to controls, a greater proportion of which were shedding MAP than would be expected if all the animals in the herds were vaccinated.

Reduced incidence of clinical JD has been shown in several studies^{12,13,17} in which the study design compared vaccinates with retrospective controls in the herd prior to adoption of vaccination programs and has also been reported in a retrospective questionnaire-based study.²⁵ Although fewer vaccinated cows were culled because of clinical JD in our study, the difference was determined to be nonsignificant on the basis of survival analysis. This may have been attributable to the overall low incidence of clinical JD in the study cattle, limiting our power to detect a significant difference. Among cows that developed clinical JD, vaccinates were in the herd a mean of 459 days longer than controls, although this difference was also not significant.

In all 3 herds of the present study, adult whole-herd prevalence of MAP shedding in feces decreased during

the years following the start of vaccination. Management changes in the herds were enacted around the same time or previous to introducing vaccination, and it should be assumed that the reduction in prevalence of MAP shedding was likely attributable to the combination of the vaccination program and other management factors. It was not a primary objective of the study to measure the effect of vaccination alone on change in the herd prevalence of MAP infection, given that most herd owners would use vaccination in combination with other disease control actions. As results of the present study demonstrate, although vaccination contributed to reduction of fecal MAP shedding and could potentially contribute to a reduction in the incidence of JD, it provides only partial protection and is not a substitute for good management. We expect that management changes in addition to vaccination will have a synergistic effect on reduction of JD in a herd. Results of a 1982 survey²⁵ of British herd owners who vaccinated cattle against MAP indicated that 86% of herds were free from clinical JD 6 years after vaccination, and the rate at which veterinarians reported decreases in the incidence of clinical JD was positively associated with management changes.

Mycobacterium avium subsp *paratuberculosis* infection status has been shown to increase risk of culling, negatively impact milk production, and extend calving-to-conception interval in cattle that test positive via bacteriologic culture of feces or ELISA.^{2,3,26,27} In the present study, positive results for culture of MAP from feces were not associated with changes in these production measures, except for calving-to-conception interval in the second lactation. The study sample size was selected to detect differences in MAP infection between vaccinates and controls (as determined on the basis of culture results), which may have limited our ability to evaluate more subtle production effects. Our sample size limitation was compounded by the high removal rate of cows in the study herds; study cohorts had up to 50% of the cows removed annually as the study progressed. Because MAP has a long incubation period before clinical JD is detected,⁴ differences in lactation performance due to the effects of subclinical MAP infection may not have been measureable in the first lactation. The second lactation may have been the only period where adequate numbers of test-positive cows remained in the herds for this difference to be observed, whereas in the third and fourth lactations, high rates of culling may have precluded detection of any significant differences.

In addition to the high rates of removal of adult cattle from the study herds, a high proportion of calves born on the farms during the period of study recruitment did not reach their first lactation because of losses due to death and culling for reproductive reasons. Thirty-eight of 200 (19%) vaccinates and 50 of 195 (26%) controls were lost prior to first lactation, which led to a lower power to detect differences than expected. Although the number of controls and vaccinates lost prior to first lactation was not significantly different, the overall loss of cohort cattle was noteworthy. Reasons for removal of these heifers were reviewed, but no important differences between groups could be discerned.

Because vaccination against MAP often results in a residual scar or swelling and vaccinated animals were required to have a unique ear tag, we were unable to mask herd owners to treatment. This was expected to have a minimal effect on the measured outcomes of the study, considering that objective measures (results of bacteriologic culture of feces, milk production, and conception records) were used to determine health outcomes.

For survival analysis of time to first positive culture result, we chose to use the continuous interval of days, although fecal samples were collected from the cows in the study on an intermittent basis at 90 days of gestation. Use of a continuous measure when cultures were not performed in a continuous manner may have led to bias and potentially overestimated the number of days to first culture result. However, the controlled design of the study, in which both experimental groups were housed and managed in the same conditions in each herd, was expected to minimize any potential difference in the time between cultures. One potential difference would be if one group had a higher rate of subclinical MAP infection and lower reproductive efficiency than the other, leading to longer intervals between 90-day pregnancy examinations and artificially extending the time to a first positive culture result. However, our analysis of reproductive measures between vaccinates and controls did not find any differences between groups in terms of calving-to-conception interval. Recurrent event Cox regression analysis was a novel method for evaluation of calving-to-conception interval across lactations, and some assumptions were made in its application. A conditional model was used where time within each risk period (lactation) was calculated from the start of lactation until the event (conception) occurred. However, this model assumed that after the event, the individual was immediately at risk for a second event. In the present study, this did not happen because there was a gap between confirmed pregnancy and the start of the subsequent lactation. This could be important if removal rates differed between vaccinate and control groups after conception was recorded, influencing which animals were at risk in the following lactation. Because the overall survival time of vaccinates and controls did not differ, the risk of bias for this analysis was considered minimal. Overall, our results indicate that vaccination against MAP vaccine may be an effective tool as part of a program to control the spread of JD in dairy cattle.

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- h. SAS, version 9.2, SAS Institute Inc, Cary, NC.

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