

Use of long-term vaccination with a killed vaccine to prevent fecal shedding of *Mycobacterium avium* subsp *paratuberculosis* in dairy herds

Cees H. J. Kalis, DVM; Jan Willem Hesselink, DVM, PhD; Herman W. Barkema, DVM, PhD; Michael T. Collins, DVM, PhD.

Objectives—To determine whether vaccination with a killed vaccine prevents fecal shedding of *Mycobacterium avium* subsp *paratuberculosis*, to compare effectiveness of a culture and cull program in vaccinated and nonvaccinated herds, and to compare paratuberculosis-related preventive management in vaccinated and nonvaccinated herds.

Sample Population—58 commercial Dutch dairy herds.

Design—Cross-sectional study (study A) in vaccinated ($n = 25$) and nonvaccinated (29) herds of dairy cows. Longitudinal study (study B) in vaccinated ($n = 2$) and nonvaccinated (2) herds of dairy cows.

Procedure—In study A, fecal samples were obtained from adult cows in herds with and without a history of vaccination with a killed vaccine. Management measures were evaluated. In study B, fecal samples were obtained 4 times at 6-month intervals from cows older than 6 months. Cows that had positive test results were removed from the herd directly after the outcome of the culture.

Results—In study A, differences were not detected among the 25 herds that were vaccinated; culture results were positive for *M avium* subsp *paratuberculosis* in 4.4% of herds. In 29 herds that had not been vaccinated, culture results were positive in 6.7%. In study B, the percentage of positive results on culture decreased from 10.9% and 5.7% to 3.5% and 0%, respectively in the 2 vaccinated herds. In the 2 nonvaccinated herds, percentages decreased from 6.1% and 16.5% to 0% and 2.3%, respectively. Management practices were different between herds that were vaccinated and herds that were not; owners of herds that were not vaccinated followed more preventive management procedures and practiced less feeding of raw milk to calves.

Conclusions and Clinical Relevance—Vaccination of calves with a killed vaccine does not prevent transmission of *M avium* subsp *paratuberculosis*; therefore, hygienic practices remain essential in herd management. (*Am J Vet Res* 2001;62:270–274)

berculosis. Paratuberculosis is a long-term, debilitating condition that often remains undetected until the onset of copious diarrhea. Clinical signs include decreased milk production, loss of body condition, and intermittent diarrhea in the absence of general signs such as fever, depression, or decreased appetite. After the onset of diarrhea, animals often become emaciated, weak, and die. The incubation period varies from 2 to 6 years. Because calves are more susceptible to infection than adults, and infected adults excrete the bacteria in large numbers in feces and milk, frequent contact between calves and cows is the most important factor in the spread of *M avium* subsp *paratuberculosis* in infected herds.¹

Culture and cull programs to control paratuberculosis have been reported to fail because the test methods used are typically unable to detect a sufficient number of infected cows.^{2,3} In most instances, however, cows that had positive test results were not removed from the herds; consequently, the management changes necessary to halt transmission of infection were not performed.^{2,3} Reintroduction of *M avium* subsp *paratuberculosis* often happens when subclinically infected cows are introduced into a herd.¹

Vaccination against paratuberculosis was first described in 1926, at which time live vaccines were used.⁴ Vaccination is effective in decreasing the incidence of clinical disease irrespective of whether live or killed vaccines are used.^{5–10} Live vaccines decrease the prevalence of shedding the organism in feces.^{5,11,12} It has been suggested that eradication of *M avium* subsp *paratuberculosis* may develop after 4 to 6 years of vaccination.^{5,13} However, despite the economic benefits, use of *M avium* subsp *paratuberculosis* vaccines has been controversial since their introduction. Disadvantages of live and killed vaccines are the development of lesions at the injection site in vaccinated calves and in humans (after being scratched by used needles), and cross-reactivity with tuberculosis tests resulting from vaccination.¹⁴ Because of the potential risk of spreading *M avium* subsp *paratuberculosis* from cattle that are vaccinated with live vaccine, and because of the possible association between *M avium* subsp *paratuberculosis* and Crohn's disease,¹⁵ only killed vaccines have been allowed for use in The Netherlands.

An important question then arises as to whether killed vaccines are as effective as live vaccines in decreasing shedding of *M avium* subsp *paratuberculosis* in feces. In 1 report regarding a herd that was vac-

Paratuberculosis in cattle is an infectious enteric disease caused by *Mycobacterium avium* subsp *paratu-*

Received Sept 13, 1999

Accepted Feb 10, 2000

From the Department of Ruminant Health, Animal Health Service, PO Box 361, 9200 AJ Drachten, The Netherlands. Dr. Collins' present address is the Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

The authors thank Esther Russchen for technical assistance.

nated with killed vaccines, shedding of *M avium* subsp *paratuberculosis* was decreased.¹⁶ However, in that experiment, the amount of shedding in the feces by vaccinated calves was not different from the amount of shedding in the feces by control calves in the same herd, and the benefits of vaccination were concluded based on a comparison of 2 herds that may have had completely different management techniques. In a controlled field study with 2 types of killed vaccine, a whole cell vaccine and a fractionated vaccine, the whole cell vaccine was most effective in preventing clinical disease and in decreasing the percentage of positive test results from intestinal tissues and bacteriologic cultures of feces.⁹ Results of 2 studies indicated that the prevalence of *M avium* subsp *paratuberculosis* in intestinal tissues of cows was not decreased by vaccination with killed vaccines.^{7,10}

Management practices to prevent transmission of infection from adult cows to calves are essential to control the spread of paratuberculosis.^{1-3,17-19} If farmers neglect preventive management practices because they rely on the use of vaccination to control the spread of infection, there may be a possible negative impact of vaccination on control of paratuberculosis.

The purposes of the study reported here were to evaluate whether vaccination with a killed *M avium* subsp *paratuberculosis* vaccine prevented fecal shedding of *M avium* subsp *paratuberculosis* in dairy herds, to compare efficacy of a culture and cull program in vaccinated and nonvaccinated herds, and to compare paratuberculosis-related preventive management practices in vaccinated and nonvaccinated herds.

Materials and Methods

Animals—The study was performed using dairy herds in the northern part of The Netherlands. For herds to be selected, > 5% of the herd, on an annual basis, had to have been affected by clinical paratuberculosis during the 2 years prior to vaccination. Vaccinated herds had a vaccination history of > 7 years. For controls, herds that had an incidence of clinical paratuberculosis similar to the vaccinated herds, of which owners had decided to control paratuberculosis without vaccination, were selected. Cattle in both studies were Holstein-Friesian and Dutch-Friesian cross-breeds. They were housed in stalls with cubicles in winter and were pastured in summer months. They were fed predried grass silage in the winter, grass in the summer, and corn silage and commercially mixed concentrates as additives. Milk production of herds varied from 7,000 to 9,000 kg/cow/lactation period. Mean herd size was 84, ranging from 27 to 240 adult cows per herd. To evaluate whether long-term vaccination against paratuberculosis prevents fecal shedding of *M avium* subsp *paratuberculosis*, results of bacteriologic cultures of feces from 25 herds after a period of at least 7 years of vaccination were compared with results of cultures of feces from 29 nonvaccinated herds. To evaluate paratuberculosis-related management practices, farmers were interviewed (study A). To evaluate efficacy of a culture and cull program in vaccinated herds, results of bacteriologic cultures of feces from 2 herds that were vaccinated annually for 7 years were com-

pared with the results of 2 nonvaccinated herds during a 2-year period. Herds remained self-contained during the study, and cows were tested for *M avium* subsp *paratuberculosis*; for cows in which test results were positive, those cows were culled immediately after receipt of the culture results (study B).

Vaccine—An experimental vaccine^a containing heat-killed *M avium* subsp *paratuberculosis* bacteria, preserved with merthiolate, suspended in a water-mineral oil suspension was used. Two milliliters of the vaccine was administered once, subcutaneously, into the dewlap at the age of 0 to 4 weeks.

Sampling procedure—Fecal samples were collected in disposable plastic examination gloves from the rectum of each cow. No lubricants were used during sample collection. After the fecal sample was collected, the glove was inverted, tied, and identified with preprinted bar-coded self-adhesive labels that documented that cow's number, as recorded in the Dutch Identification and Registration system.²⁰ Samples were stored at 4 C during transport and processed for isolation of *M avium* subsp *paratuberculosis* within 24 hours after arrival at the laboratory. In study A, cows > 24 months old were sampled once. In study B, cows > 6 months old were sampled 4 times at 6-month intervals. In the event of contaminated samples, cows were sampled and cultures were performed a second time.

Laboratory analysis—Fecal samples were examined by use of a bacteriologic culture technique with a modified Jørgensen method.²¹ A teaspoon of feces weighing approximately 2 g was used. Fecal samples were decontaminated for 30 minutes with 8 ml 4% NaOH solution, followed by centrifugation (1,000 × g), and then 30 minutes of exposure to 5 ml of a mixture of oxalic acid (5 mg/ml) and malachite green (1 mg/ml). After centrifugation (1,000 × g), the decontaminated sediment was resuspended and incubated overnight in 4 ml of a mixture of neomycin (0.5 mg/ml) and amphotericin B (50 mg/ml) solution. The separation layer between the lower layer of particulate matter and the upper layer of clear antibiotic solution was inoculated onto modified Löwenstein-Jensen agar slants. The tubes were inspected at 8, 12, 16, and 26 weeks of incubation for evidence of *M avium* subsp *paratuberculosis* growth. Results were considered positive if 1 or more colonies were identified as *M avium* subsp *paratuberculosis* in 1 or more culture tubes. A culture tube was recorded as contaminated if fungal or bacterial growth other than *M avium* subsp *paratuberculosis* was found. An entire sample was considered to be contaminated if > 3 tubes were found to be contaminated.

Management analysis—The following management practices, considered to be the most important factor in the prevention of transmission of *M avium* subsp *paratuberculosis*,^{1,3} were studied: use of a separate calving parlor; this was defined as a parlor used exclusively for calving, separated by a concrete wall from the barn with the other cattle. The parlor had to be cleaned thoroughly after each calving, and the calf had to be removed

Table 1—Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (as detected by bacteriologic culture) in cows after long-term vaccination against paratuberculosis, compared with nonvaccinated herds

Herds	Vaccination period	No. herds	No. cows	No. cows infected (%)	Range per herd (%)
Vaccinated	7–13	25	2,193	94 (4.3)	0–22
	7	4	367	16 (4.4)	0–8
	8	6	489	151 (7.0)	0–22
	9	1	37	152 (8.1)	N/A
	10	3	254	153 (3.9)	3–5
	11	5	420	154 (1.7)	0–3
	12	5	529	155 (5.1)	0–7
	13	1	97	0	N/A
	Nonvaccinated	N/A	29	2,259	151 (6.7)

N/A = Not applicable.

from the dam immediately after birth; milk feeding management; this was defined as the exclusive use of colostrum from each calf's dam; thereafter, use of commercial milk replacers was acceptable; separate housing; this was defined as housing calves in barns separate from those of adults; and separate pasture; this was defined as pasture of calves on plots separate from, and not used by, older cows, and fertilized exclusively with synthetic fertilizers.

Data analyses—Assessment of management practices between vaccinated and nonvaccinated herds were compared by use of the χ^2 test on contingency tables.²² Because the number of colony-forming units (CFU) of *M avium* subsp *paratuberculosis* was obtained from 2 g of feces, the numbers of CFU had to be divided by 2 to give the number of CFU per gram. The number of CFU in samples with positive culture results from vaccinated and nonvaccinated herds was compared after categorization at a cutoff value of 10 CFU (total colonies in all inoculated tubes) by use of χ^2 analysis on contingency tables. Prevalence of *M avium* subsp *paratuberculosis* in vaccinated and nonvaccinated herds was compared by use of a 2-tailed *t*-test.²² Values of *P* < 0.05 were considered significant.

Results

Culturing of feces (study A)—From 2,193 fecal samples obtained from 25 herds that had been vaccinated, 97 (4.4%) samples yielded positive bacteriologic culture results (Table 1). In 6 herds, *M avium* subsp *paratuberculosis* did not grow in culture. In the 19 herds in which *M avium* subsp *paratuberculosis* did grow, the prevalence of infection ranged from 2 to 29%, with a mean prevalence of 5.8%.

From 2,259 fecal samples obtained from 29 nonvaccinated herds, 151 (6.7%) samples yielded positive bacteriologic culture results (Table 1). In 4 herds, *M avium* subsp *paratuberculosis* did not grow in culture. Prevalence of *M avium* subsp *paratuberculosis* (in the 25 herds from which positive results were obtained) ranged from 1 to 20%, with a mean prevalence of 7.8%. Prevalence of *M avium* subsp *paratuberculosis* in the 25 vaccinated herds was not significantly different from the prevalence in the 29 nonvaccinated herds (*P* = 0.27).

Culturing of feces (study B)—After the onset of the culture and cull program, the percentage of cows

with positive culture results in 1 of the 2 vaccinated herds (herd size, 322 cows > 6 months old) decreased from the first herd sampling to the second, from 10.9 to 3.2%, respectively. This prevalence of positive culture results remained fairly constant during subsequent samplings of herds, with 2.7 and 3.5% at the third and fourth sampling times, respectively. The percentage of cows with positive culture results in the second vaccinated herd (124 cows > 6 months old) fluctuated between samplings, with a decrease from 5.7% at first sampling to 2.8% at second sampling, an increase to 9.1% at third sampling and a decrease to 0% at fourth sampling.

In 1 of the 2 nonvaccinated herds (98 cows > 6 months old), the percentage of cows with positive culture results remained fairly constant between first and second samplings (6.1 and 6.4%, respectively), followed by a sharp decline to 1.1 and 0% in the third and fourth samplings. In the second nonvaccinated herd (79 cows > 6 months old), the percentage of cows with positive culture results fluctuated between samplings with 16.5% at first sampling, 1.3% at second sampling, 9.1% at third sampling, and 2.3% at fourth sampling.

Vaccinated cows excreted a slightly lower number of bacteria, compared with nonvaccinated cows; 75% of the positive results obtained by culture of fecal samples of vaccinated cows contained < 10 CFU (< 5 CFU/g), compared with 56% of the positive results on culture of feces from nonvaccinated cows containing < 5 CFU/g (*P* = 0.07).

Management practices (study A)—In 8 (32%) vaccinated herds and 14 (48%) nonvaccinated herds, a separate calving parlor was used and calves were separated immediately after birth. In 3 (12%) vaccinated herds and 23 (79%) nonvaccinated herds, calves were fed colostrum only from their dams and were fed no raw milk after the colostrum period. Young stock < 12 months of age were housed in a separate barn in 14 (56%) vaccinated herds and in 20 (69%) nonvaccinated herds. Calves put out on pasture on grass contaminated with manure of adult cows was avoided in 4 (16%) of the vaccinated herds and in 9 (31%) of the nonvaccinated herds. None of the producers of the vaccinated herds performed all 4 preventive management practices; 5 (17%) producers of the nonvaccinated herds performed all 4 preventive management practices.

Discussion

Long-term vaccination did not eliminate infection with *M avium* subsp *paratuberculosis* from commercial dairy herds. Results of the cross-sectional study (study A) revealed that the rate of shedding of *M avium* subsp *paratuberculosis* in feces was not significantly different between the vaccinated (4.4%) and nonvaccinated herds (6.7%; Table 1). This result was unexpected and was in contrast with the data previously reported by Argente.⁵ Argente⁵ compared 2,073 vaccinated and 1,281 nonvaccinated cattle that were born during a control program, which was based on vaccination and hygiene, and reported there was an 85% decrease in the rate of shedding in feces in the vaccinated cattle and only a 23% decrease in the rate of shedding in nonvaccinated cattle born in the same period and in the same herds. Use of live vaccine in that study may explain the greater effect of vaccination on rate of shedding of *M avium* subsp *paratuberculosis* in feces. Larsen et al⁹ revealed that vaccination with a killed whole cell vaccine, as was used in our study, decreased the percentage of shedding from 11% to 5% in vaccinated and control calves in the same herds. The absence of reduction of culture-positive fecal samples in Larsen's study and the absence of reduction of the percentage of culture positive autopsies in earlier studies^{7,10} may be attributable to a combination of lower efficacy of killed vaccines and opposing effects of neglecting appropriate preventive management practices. Alternatively, the difference in results obtained in our study, compared with Argente and Larsen, also may have been attributable to the insensitivity of the culture method they used on the feces. Both used the Merkal sedimentation technique with benzylkonium chloride as the decontaminant.

In the longitudinal study (study B), vaccination may have caused the decreased rate of shedding of *M avium* subsp *paratuberculosis* in the feces from 10.9 and 5.7% to 3.5 and 0%, respectively, in the 2 herds during an 18-month period. However, the rate of shedding also decreased from 6.1 and 16.5% to 0 and 2.3%, respectively, in the 2 nonvaccinated herds. Consequently, the decreased rate of shedding we observed cannot be attributed solely to use of the killed vaccine. Decrease in shedding rates of *M avium* subsp *paratuberculosis* in herds that had been vaccinated with live vaccine and were involved in a culture and cull program was also observed by Jørgensen¹¹; however, the decrease in shedding rates may have been falsely attributed to the use of vaccine because no suitable cohort controls were available. The observed fluctuation in the percentage of cows that were shedding *M avium* subsp *paratuberculosis* in the herds of the present study emphasizes the importance of repeating cultures of feces from the entire herd before a herd can be declared free of paratuberculosis.

Farm management practices that limited the opportunities for transmission of *M avium* subsp *paratuberculosis* played as important a role in decreasing the rate of shedding the bacteria in feces as did vaccination. Owners of nonvaccinated herds followed more of the preventative measures against infection than did owners of vaccinated herds. Of the practices

that were monitored, the frequency of feeding raw milk from cows to calves was substantially higher in vaccinated herds than in nonvaccinated herds. Vaccination may not have provided sufficient protection to overcome the constant exposure of calves to *M avium* subsp *paratuberculosis* via milk. This may explain why the rate of fecal shedding was not different between vaccinated and nonvaccinated herds and further supports the idea that herd owners who are vaccinating against paratuberculosis depend on the vaccine to control the infection and ignore other control measures that are necessary to prevent infection.

Two years of a culture and cull program, which consisted of culturing feces of each herd at 6-month intervals, was effective in achieving a decreased prevalence of *M avium* subsp *paratuberculosis* in vaccinated and nonvaccinated herds. Culling of cows that had positive culture results, combined with prevention of infection through proper calf management practices appeared at least as, if not more, important than vaccination for control of paratuberculosis, because no extra benefit of vaccination was found in the herds described here. The number of herds was limited, but to the authors' knowledge, this is the first study to contrast the effects of vaccination with suitable controls on a herd basis and to monitor other control measures for paratuberculosis used in the herds. Studies of larger numbers of herds are indicated before results of this study can be generalized.

*Paratuberculose Vaccin, ID-Lelystad, Lelystad, The Netherlands.

References

1. Sweeney RW. Transmission of paratuberculosis. *Vet Clin North Am Food Anim Pract* 1996;12:305-313.
2. Benedictus G. Evaluation of organized control of bovine paratuberculosis in Friesland province, The Netherlands. *Tijdschr Diergeneeskd* 1984;109:905-916.
3. Reinders JS. The control of paratuberculosis. *Tijdschr Diergeneeskd* 1986;111:426-430.
4. Vallée H, Rinjard P. Etudes sur l'enterite paratuberculeuse des bovines. *Rev Gen Med Vet* 1926;35:1-9.
5. Argent G. Efficiency of vaccination and other control measures estimated by fecal culturing in a regional program, in *Proceedings*. 3rd Int Colloq Paratuberculosis 1991;495-498.
6. Huitema H. Paratuberculosis in cattle and vaccination against this disease. *Tijdschr Diergeneeskd* 1968;93:953-963.
7. Kalis CHJ, Benedictus G, van Weering HJ, et al. Experiences with the use of an experimental vaccine in the control of paratuberculosis in The Netherlands, in *Proceedings*. 3rd Int Colloq Paratuberculosis 1992;484-492.
8. van Schaik G, Kalis CHJ, Benedictus G, et al. Cost-benefit analysis of vaccination against paratuberculosis in dairy cattle. *Vet Rec* 1996;139:624-627.
9. Larsen AB, Moyle AI, Himes EM. Experimental vaccination of cattle against paratuberculosis (Johne's Disease) with killed bacterial vaccines: a controlled field study. *Am J Vet Res* 1978;39:65-69.
10. Wentink GH, Bongers JH, Zeeuwen AA, et al. Incidence of paratuberculosis after vaccination against *M. paratuberculosis* in two infected dairy herds. *Zentralbl Veterinärmed* 1994;41:517-522.
11. Jørgensen JB. The effect of vaccination on the excretion of *Mycobacterium paratuberculosis*. In: Jørgensen JB, Aalund O, eds. *Paratuberculosis, diagnostic methods, their practical application and experience with vaccination*. EUR 9000 EN, 1984;131-136.
12. Stuart P. Vaccination against Johne's disease in cattle exposed to experimental infection. *Br Vet J* 1965;121:289-318.
13. Wilesmith JW. Johne's disease: a retrospective study of vaccinated herds in Great-Britain. *Br Vet J* 1982;138:321-330.

14. Stabel JR, de Lisle GW, Sockett DC. Debate: paratuberculosis control. Vaccination vs. eradication by on-farm infection control and test-and-slaughter, in *Proceedings*. 5th Int Colloq Paratuberculosis 1996;212-222.
15. Chiodini RJ, Rossiter CA. Paratuberculosis: a potential zoonosis? *Vet Clin North Am Food Anim Pract* 1996;12:457-467.
16. Kormendy B. The effect of vaccination on the prevalence of paratuberculosis in large dairy herds. *Vet Microbiol* 1994;41:117-125.
17. Goodger WJ, Collins MT, Nordlund KV, et al. Epidemiologic study of on-farm management practices associated with prevalence of *Mycobacterium paratuberculosis* infections in dairy cattle. *J Am Vet Med Assoc* 1996;208:1877-1880.
18. Johnson-Ifearegulu YJ, Kaneene JB. Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA, dairy herds. *Prev Vet Med* 1998;37:41-54.
19. Sockett DC. Update on control and management of Johne's disease. *Bovine Pract* 1994;28:136-138.
20. Nielen M, Jansen FCM, van Wuijckhuise LA, et al. Dutch cattle identification and registration (I&R) system: analysis of its use for controlling an outbreak of foot and mouth disease. *Tijdschr Diergeneeskd* 1996;121:576-581.
21. Kalis CHJ, Hesselink JW, Russchen EW, et al. Factors influencing the isolation of *Mycobacterium avium* subsp. *paratuberculosis* from bovine fecal samples. *J Vet Diagn Invest* 11:345-351.
22. Rothman KJ. *Modern epidemiology*. Boston: Little, Brown & Co, 1986.