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Effect of management practices on paratuberculosis prevalence in Danish dairy herds

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Abstract

A voluntary risk-based control program on paratuberculosis in dairy cattle was initiated in Denmark in 2006. Cows were categorized as high-risk (antibody-positive at least once within the last 3 tests) or low-risk animals based on the results of 3 to 4 annual milk ELISA detecting *Mycobacterium avium* ssp. *paratuberculosis* (MAP)-specific antibodies. High-risk animals require management practices aimed at decreasing calf exposure to MAP-contaminated colostrum and milk, and feces originating from these cows. Moreover, repeated test-positive cows are recommended for slaughter before next calving. The objective was to assess the effect of different management practices on the prevalence of MAP-specific antibodies. A questionnaire on management practices was distributed to 1,261 participating herds in December 2008. A total of 1,092 (87%) herd managers returned the questionnaire. Repeated prevalence data from 1,081 herds were available for a period up to 4.25 yr after the first test round. The changes in the prevalence of MAP-specific antibodies from the start of interventions were assessed using a hierarchical logistic model, where different management practices were assessed: a) culling of repeated test-positive cows, b) separation of high-risk from low-risk cows in calving areas, c) cleaning of calving areas after high-risk cows calved, d) removal of calves born to high-risk dams within 2 h after calving, e) use of colostrum for feeding of heifer calves from low-risk cows only, f) use of waste milk for feeding of heifer calves from low-risk cows only, g) herd size, and h) proportion of purchased animals. Multivariable analyses suggested that only the proportion of purchased animals (>15% purchased animals as well as 0 to 15% purchased animals compared with no purchased animals in the herd), culling of repeated test-positive animals, and use of waste milk from specific cow groups influenced the decrease in prevalence of MAP-specific antibodies. The control program has been running for just 4.25 yr, and it is assumed that the full effect of the risk-based management practices will only be observed after 4 to 8 yr. Therefore, lack of association between some practices and decrease in prevalence may be a reflection of a short study period. Furthermore, decreases in the prevalence of MAP-specific antibodies may not reflect discontinued transmission of MAP in all age groups.

Introduction

Paratuberculosis in cattle is a chronic infection caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**). *Mycobacterium avium* ssp. *paratuberculosis* infections are widespread among dairy cattle in Europe (Nielsen and Toft, 2009), and cause significant economic losses (Ott et al., 1999). These losses are primarily due to decreased value at slaughter (Kudahl and Nielsen, 2009), decreased milk yield, premature culling (Raizman et al., 2009), and indirect effects thereof (e.g., increased replacements, resulting in a younger herd, which produces less milk; Kudahl et al., 2007). Therefore, several control programs have been established to decrease the spread of MAP (Benedictus et al., 2000; Kennedy and Nielsen, 2007). Some programs have terminated, and reasons for giving up are rarely reported.

Lack of valid diagnostic tests to detect MAP-infected animals and lack of compliance with recommended practices could be possible reasons.

Mycobacterium avium ssp. *paratuberculosis* infections are thought to occur predominantly in calfhood (Windsor and Whittington, 2010), although replacement cattle raised in presumed uninfected herds have been found infected, suggesting that adult infections do occur (Wells et al., 2010). Cell-mediated immune responses are initiated to keep MAP numbers low in infected tissues, and the infection remains latent. At some point in time, the humoral immune responses take over, and the animal loses control of the infection (Coussens, 2001). Infected animals most likely lose control of the infection between 2 and 6 yr of age (Nielsen and Ersbøll, 2006). Loss of control is usually accompanied by occurrence of IgG1-antibodies in blood and milk, and shedding of significant numbers of MAP in feces. Therefore, detection of antibodies in serum or milk can be used as tests for diagnosis of the loss of control condition or animals infectious with MAP. No ante-mortem test can accurately detect MAP-infected animals (Nielsen and Toft, 2008).

Transmission of MAP is primarily thought to occur via the fecal-oral route, although infections in utero and via ingestion of MAP-contaminated colostrum or milk are likely routes of transmission (Sweeney, 1996). Calves are considered more susceptible to infection than are adults, although animals of all ages are at risk of becoming infected (Windsor and Whittington, 2010). Therefore, efforts minimizing calf exposure to feces, colostrum, and milk from MAP infectious adult animals should decrease the incidence of MAP infections. Numerous risk factor studies confirmed some risk factors (e.g., lack of cleaning of maternity pen after each use, Johnson-Ifearulundu and Kaneene, 1998; and feeding of colostrum from multiple cows, Nielsen et al., 2008); however, the long and unpredictable incubation period make risk factor studies challenging. Consequently, simulation studies were used to study control strategies, and most findings suggest that focus should be given to management practices that decrease transmission, possibly in combination with test-and-cull procedures. Test-and-cull procedures alone may not decrease the prevalence (Groenendaal et al., 2003; Kudahl et al., 2007). Therefore, the Danish control program on MAP focuses on a combination of test-and-manage and test-and-cull practices.

The Danish control program on bovine MAP was initiated in March 2006 (Nielsen et al., 2007). The program is risk-based, and animals are divided into high-risk and low-risk groups, based on 4 annual herd screenings using a milk antibody ELISA (Nielsen, 2009a). High-risk cows are defined as animals with at least 1 ELISA-positive result among the last 4 test-results, whereas low-risk cows are those that are test-negative in the last 4 tests. Furthermore, high-risk animals are divided into red and yellow cows, where red cows have had at least 2 positive test results out of the last 4 tests and yellow cows have had other test combinations based on repeated results. Low-risk cows are denoted as green, and considered noninfectious, whereas red and yellow cows are potentially infectious. Details about the classification scheme are provided in Nielsen (2009b).

The degree to which specific management practices aimed at breaking transmission routes actually lead to a decrease in the prevalence of MAP remains to be demonstrated. Thus, the objective was to assess and compare changes in test prevalence of MAP-specific antibodies in Danish dairy herds following different management practices.

Materials and Methods

Participants and Recommendations

The number of participants in the Danish control program increased from 621 in July 2006 to 1,261 at the end of 2008 (Nielsen, 2009b). Participating farmers are advised to decrease the risk of

transmission through the following recommendations: red cows should be slaughtered before next calving so they do not enter the calving area; high- and low-risk cows should calve in separate calving areas, and the calving area must be cleaned after the calving of high-risk cows; calves born to high-risk cows must be removed as soon as possible and preferably within 2 h after birth; and milk and colostrum from high-risk cows must not be used for feeding of heifer calves staying in the production system for more than 1 yr.

Management Practices

Mailed questionnaires were sent to the 1,261 herds participating on Dec. 23, 2008. Reminders were sent twice to the nonresponders (sent on Jan. 22, and Mar. 1, 2009). The questionnaire included a 1-page set of questions, including questions pertaining to how the farmer followed the recommendations to decrease transmission, culling practices, and the purpose of participation. The questionnaire included 15 close-ended questions, each having 3 options [yes, no, and do not know (Table 1)].

Table 1. Questions included in mailed questionnaire to 1,261 farmers participating in the Danish control program on paratuberculosis in dairy herds. The number of responses in each category from the 1,092 responders is given in the last three columns

Management factor[1]	Yes	No	Do not know
Milk and Colostrum feeding to heifer calves			
- We use colostrum from Red cows	67	970	44
- We use colostrum from Yellow cows	310	707	64
- We use colostrum from Green cows	1009	32	40
- We use waste milk from Red cows	90	949	42
- We use waste milk from Yellow cows	286	742	53
- We use waste milk from Green cows	745	293	43
Calvings			
- Most Red cows calve again	178	871	31
- A few Red cows calve again	875	172	34
- Red cows calve separated from Green cows	539	504	38
- Red cows calving pen cleaned between calvings	411	609	61
- Yellow cows calve separated from Green cows	336	700	45
- Yellow cows calving pen cleaned between calvings	297	752	32
- Calves born to Red cows are removed within 2 h	903	128	49
- Calves born to Yellow cows are removed within 2 h	736	291	54
- Calves born to Green cows are removed within 2 h	446	580	55

[1]"Red", "Yellow" and "Green" cows are categories of animals based on their test-results, where Red cows were repeated antibody positive, Green cows antibody-negative on up to last 3 tests, and Yellow cows with other test-combinations based on repeated results. The response pertained to the general practice on the date of response by the farmer

The questionnaire was developed based on interviews with 16 farmers in a pilot study in October 2006, where they were asked open-ended questions about their management practices. A new questionnaire based on these interviews were tested on the same 16 farmers in April to May 2007, and subsequently used by 35 herd health consultants in 99 herds as a tool to evaluate management practices. An English translation of the questionnaire is in Table 1. The farmers were instructed to

answer the questions on their management practices on the day the questionnaire was filled in. Prior to receiving results of herd screenings (see later), farmers could not divide animals into high- and low-risk animals, because this categorization of animals had not been established. It was assumed that farmers performed the management practices from the date of the first test-round, because they had been doing a risk-assessment with their herd health consultant around this date. These data were self-reported and were not validated to determine if farmers were actually implementing the management plan.

The responses to the questions were reorganized into 6 categorical variables used as independent variables in the analyses. These were

1. Red cow calvings: a) no red cows calve again, b) some red cows calve again, or c) most red cows calve again.

2. Separation of calves from high-risk dams: a) calves are separated from red and yellow dams within 2 h, b) calves are separated from red dams, but not yellow dams, within 2 h, or c) calves are not separated from high-risk dams within 2 h after birth.

3. Cleaning of calving area: a) calving area is cleaned after red and yellow calvings, b) calving area is cleaned after red, but not yellow calvings, or c) calving area is not cleaned after high-risk calvings.

4. Separation of high- and low-risk dams in calving area: a) red and yellow cows separated from green cows in calving area, b) red, but not yellow cows, separated from green cows in calving area, or c) no separation of high- and low-risk cows in calving area.

5. Use of colostrum: colostrum for feeding of heifer calves used from: a) green cows only, b) yellow and green cows, but not red cows, or c) all cows.

6. Use of waste milk: waste milk used for feeding of heifer calves occurs from: a) green cows only, b) yellow and green cows, but not red cows, or c) all cows.

Some farmers responded do not know to some questions. They were considered not to have established procedures to avoid transmission and were classified as worst-case scenarios. The distribution of responses to the questionnaire data are in Table 1. A total of 1,092 herds (87%) provided responses, but of these, 11 had ceased production after the questionnaire data were obtained. Therefore, 1,081 herds provided both questionnaire and prevalence data.

Additionally, the total herd size and number of purchased animals (defined as animals currently in, but not born in the herd) on the first test date in each herd was obtained from the Danish Cattle Database (Knowledge Centre for Agriculture, Danish Agricultural Advisory Service, Aarhus N, Denmark). The herds were divided into 3 equally sized groups based on herd size: a) <120 cows, b) 120 to 165 cows, and c) >165 cows. Four purchase groups were created (using the proportion of purchased animals in the herd, i.e., number of purchased animals divided by total herd size, at the first test date): 1) no purchased animals, 2) 0 to 5% purchased animals, 3) 5 to 15% purchased animals, and 4) >15% purchased animals in the herd.

Prevalence Estimation

Herds in the control scheme were tested by antibody ELISA through the milk recording scheme, where milk samples are obtained 6 or 11 times per year in each herd. Four of these test rounds were used for prevalence estimation at 4 annual sampling points. Because milk samples were used, only lactating animals were tested. An in-house ELISA (Nielsen, 2002) was used until October 15, 2008,

and a commercial test (IDScreen, ID-Vet, Montpellier, France) was used after that date. The in-house test was considered positive at an optical density reading of 0.3, and the commercial test was considered positive at a sample-to-positive ratio of 0.2. These thresholds were chosen to make the tests as sensitive as possible, potentially resulting in more false positives. The in-house ELISA should be able to detect >70% of the infectious animals on the date they became infectious (Nielsen, 2008), whereas the commercial test should be able to detect >90% (Nielsen, unpublished data). The in-house test had an estimated specificity of 93%, but was greatly age-dependent (Nielsen and Toft, 2006). The specificity of the commercial test was >99% (S. S. Nielsen, unpublished data).
The ELISA data for prevalence estimation were obtained from the Danish Cattle Database on June 6, 2010, encompassing the entire program period. At each test date, the prevalence was determined as the number of ELISA-positive cows among animals tested. Subsequently, a time variable (**TIME**) was created, indicating the time from the first test date (i.e., the start date in the program) to a specific test date. Unfortunately, the initial explorative descriptive analyses, examining prevalence of MAP-specific antibodies as a function of TIME, overall and stratified by tests, showed that the apparent prevalence of MAP-specific antibodies in the 2 tests differed to an extent, which discouraged further modeling of the full data set. Because of the significantly lower specificity and sensitivity of the in-house test compared with the commercial test, apparent prevalence estimates were not comparable. Furthermore, using a conversion factor or calculation of true prevalence for the entire period could introduce biases for which there is no control. Therefore, only test results of the second test (IDScreen, ID-Vet) were used for further analyses.
The 1,081 herds contributed with a total of 5,677 herd examinations, with 901,814 cow tests using the IDScreen test. Twenty-six herds were tested 1 time, 22 herds 2 times, 30 herds 3 times, 95 herds 4 times, 407 herds 5 times, 413 herds 6 times, 80 herds 7 times, and 8 herds 8 times. This reflects that herds joined the program throughout the period from March 2006 to June 2010, and, therefore, have participated for different lengths of time, resulting in different number of samples. Among the 5,677 herd examinations, the distribution of animals tested at each herd examination was as follows: minimum = 8 cows; twenty-fifth percentile = 105 cows; median = 142 cows; seventy-fifth percentile = 190 cows; and maximum = 1,225 cows.

Descriptive Statistics

A semi-parametric analysis was performed using the GAM procedure in SAS version 9.2 (SAS Institute, Cary, NC), using the prevalence of MAP-specific antibodies as the outcome and TIME, stratified by each of the 8 risk factors (one at a time), as the covariate modeled with a univariate smoothing spine with 4 degrees of freedom. These analyses were used to explore the relationship between prevalence of MAP-specific antibodies and TIME. The analyses suggested, based on visual inspections, that an approximately linear relationship could be used for all risk factors, although indications of an initial lag phase were suggested for some risk factors.
Furthermore, 2-way cross-tabulations of the 8 risk factors were made. Some combinations of outcomes were not possible (e.g., feeding colostrum from red dams if these did not calve again).

Statistical Analyses

To account for the clustering within herd, a logistic analysis using a generalized linear mixed model approach was carried out. Essentially, the initial model for each risk factor was

$$\text{logit}(p) = a + RF + TIME + TIME \times RF + Herd,$$

where p is the probability of testing positive in a herd (modeled using the number of positive/number tested per herd per test round), a is the common

intercept, RF is the fixed effect of one of the 8 potential risk factors, TIME is the time from start in the program, and Herd is a first-order auto-regressive [AR(1)] correlation structure used for the working correlation of the generalized estimating equations analysis to account for clustering of repeated measures within farm. For each analysis, the main effect of the risk factor was assessed and removed if nonsignificant ($P > 0.05$), whereas the interaction term was kept even if it was deemed nonsignificant and results were reported as estimates of the slope of the linear function of TIME for each of the strata of the risk factor, with a *P*-value reflecting the test of significance of the interaction. Furthermore, confounding was evaluated by assessing if parameter estimates changed by more than 20% by including and excluding each risk factor.

The analyses were carried out using the GENMOD procedure in SAS version 9.2. First, univariable analyses were carried out. Subsequently, a forward selection procedure was carried out on all risk factors to establish a multivariable model. Two-way interaction terms between the risk factors and TIME were evaluated in the final model. The results were presented as parameter estimates, but were plotted for the final multivariable model to illustrate the interdependence between risk factors. But, in this plot, only predictions supported by more than 40 herds in each category were included to simplify the graph.

Results

The estimates from the univariable analyses are given in Table 2. To illustrate the implications of the differences between the estimated slopes, consider that a slope of −0.18 corresponds to an odds ratio (**OR**): OR = exp(−0.18) = 0.84 for a 1-yr period or OR = exp(−0.18 × 4) = 0.48 for 4 yr, which implies that for herds without purchase, the prevalence was approximately halved in 4 yr. Another example for herds in which most red dams calve again compared with those in which no red dams calve again, OR = exp(−0.04 × 4) = 0.85 (i.e., approximately a 15 percentage point prevalence decrease in a 4-yr period. For the multivariable analysis, only the risk factors regarding whether red cows calve again, purchase group, and the use of waste milk for feeding were included in the final model. No significant interactions between these risk factors were found, and parameter estimates of the factors in the resulting model did not change noticeably by including other factors. The results of the multivariable analyses are in Table 3. Only 8 of the 27 possible combinations were represented by more than 40 herds. The predictions for these 8 combinations are in Figure 1. For all models, the estimated autocorrelation was approximately 0.83, suggesting a strong within-herd effect on the prevalence (i.e., a herd with a higher-than-expected probability of cows testing positive at TIME = 1 will most likely also have a higher-than-expected probability of cows testing positive at TIME = 2). Still, the correlation between 2 observations decreases, as the time between observations decreases, from 0.83 between successive measurements to $0.83^2 = 0.69$ and $0.83^3 = 0.57$ when measurements are 2 and 3 time points apart, respectively.

Table 2. Results of univariable logistic analyses of the probability of testing positive in *Mycobacterium avium* subsp. *paratuberculosis* specific antibody ELISA for Danish dairy cows tested at different time points (TIME) after first test-results were available in herds with different management practices

Risk factor[1]	**N_{Herds}**	**Estimate**[2]	**SE**	***P*-value**[3]
Intercept (all models)		-2.54	0.05	<0.001
Red cows calve again				
Most	209	-0.04	0.02	0.014
Some	717	-0.10	0.02	
None	155	-0.14	0.03	
Separation of cows in calving area				
No separation	467	-0.10	0.02	0.69
Red from Green	279	-0.09	0.02	
Red & Yellow from Green	335	-0.09	0.02	
Calves removed within 2 h from				
No cows	156	-0.08	0.03	0.015
Red cows	190	-0.15	0.03	
Yellow and Red cows	735	-0.09	0.02	
Calving area cleaned after				
No cows	582	-0.08	0.02	0.181
Red cows	219	-0.12	0.02	
Yellow and Red cows	280	-0.10	0.02	
Colostrum used from				
Red, Yellow & Green cows	107	-0.06	0.03	0.554
Yellow & Green cows	267	-0.09	0.02	
Only Green cows	707	-0.10	0.02	
Waste milk used from				
Red, Yellow & Green cows	132	-0.09	0.03	0.021
Yellow & Green cows	209	-0.14	0.02	
Only Green cows	740	-0.09	0.02	
Herd size				
> 165 cows	361	-0.07	0.02	0.002
121 to 165 cows	355	-0.12	0.02	
< 121 cows	365	-0.14	0.02	
Purchase of animals				
> 15% of herd	357	-0.05	0.02	<0.001
5 to 15% of herd	208	-0.11	0.02	
0 to 5% of herd	249	-0.11	0.02	
No purchased animals in herd	457	-0.18	0.03	

[1]"Red", "Yellow" and "Green" cows are categories of animals based on their test-results, where Red cows were repeated antibody positive, Green cows antibody-negative on up to last 3 tests, and Yellow cows with other test-combinations based on repeated results. [2]The estimate for each stratum represents the slope of TIME variable within that stratum.[3]The *P*-value gives the significance of the interaction (i.e., different slopes between strata), the main effect of TIME was highly significant in all analyses.

Table 3. Results of multivariable logistic analyses of the probability of testing positive in *Mycobacterium avium* subsp. *paratuberculosis* specific antibody ELISA for Danish dairy cows tested at different time points (TIME) after first test-results were available in herds with different management practices

Risk factor[1]	**Estimate**[2]	**SE**	***P*-value**[3]
Intercept	-2.54	0.05	<0.001
Red cows calve again			
Most	0.11	0.03	0.009
Some	0.04	0.02	
None	0.00	0.00	
Waste milk used from			
Red, Yellow & Green cows	0.00	0.00	0.021
Yellow & Green cows	-0.03	0.03	
Only Green cows	0.03	0.02	
Proportion purchased animals in herd			
> 15% of herd	-0.12	0.04	<0.001
0 to 15% of herd	-0.19	0.04	
No purchased animals in herd	-0.25	0.04	

[1]"Red", "Yellow" and "Green" cows are categories of animals based on their test-results, where Red cows were repeated antibody positive, Green cows antibody-negative on up to last 3 tests, and Yellow cows with other test-combinations based on repeated results. [2]The estimate for each stratum represents the slope of TIME variable within that stratum. [3]The *P*-value gives the significance of the interaction (i.e., different slopes between strata), the main effect of TIME was highly significant in all analyses.

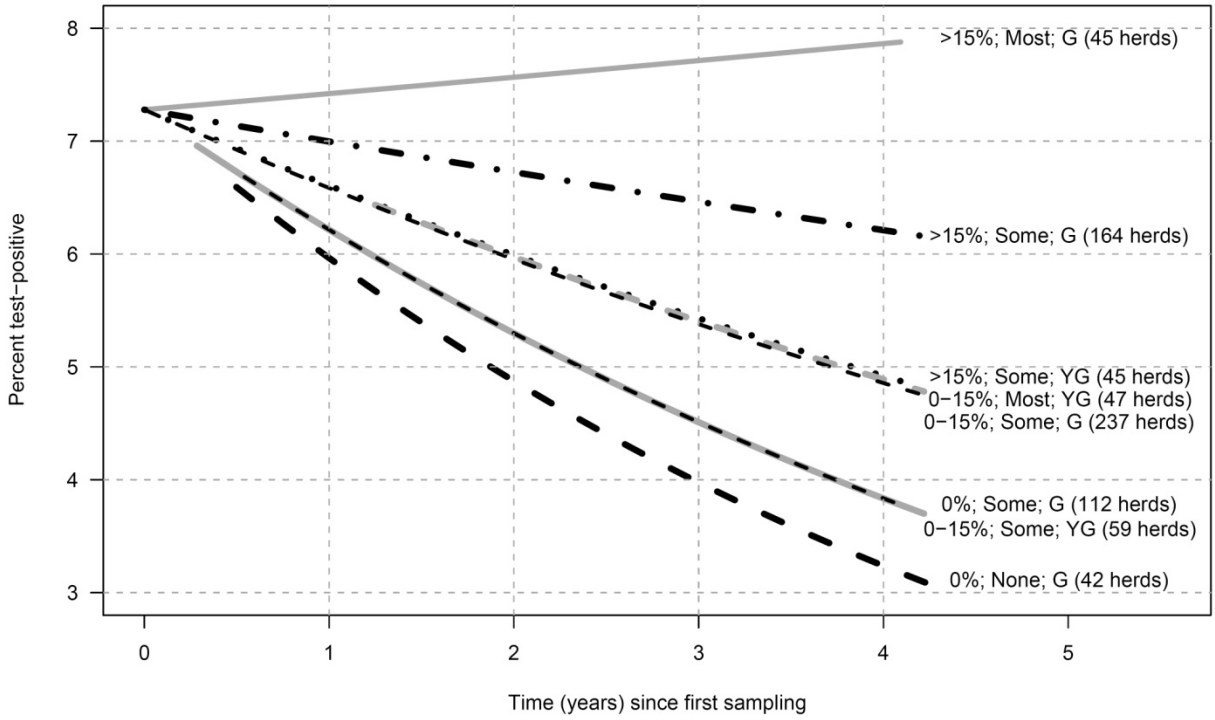

Figure 1. Illustration of the multivariable association between test-prevalence and TIME stratified by the purchase, waste milk, and removal policies. Only the 8 most common combinations of practices with more than 40 herds are illustrated. For each line, the proportion of purchased animals (0%, 0 to 15% or > 15%), the policy for removal of Red cows (Most, Some or None), the waste milk usage (G = green only, YG = yellow and green cows), and number of herds practicing the strategy is given.

Discussion

A decrease in the prevalence of MAP infections usually occurs via changes in management, and not test-and-cull schemes alone (Groenendaal et al., 2003; Kudahl et al., 2007). The theoretical basis for the lack of an effect of a test-and-cull strategy is primarily that the incidence in seroconversion is relatively stable from 2 to 6 yr of age (i.e., new test-positives will quickly replace culled test-positives). The results from this study suggest that the stated intention to cull repeated test-positive animals (red cows) does contribute significantly to a decrease in the prevalence and that the culling effect may be attributed to the high test frequency used in the present control program, whereby infectious animals are quickly identified and removed from the herd before they shed high numbers of MAP into the environment. Furthermore, a general assumption often used in simulation models is that antibodies are generally considered to occur after the animal is shedding MAP (Sweeney et al., 2006). But, the animals classified simply as shedders may not be infectious, and, therefore, the simulation models may not address this aspect appropriately. The test strategy used in the Danish program should enable detection of most infected animals before they become infectious (Nielsen, 2008), thereby enabling their removal from the herd before they infect susceptible animals.

The proportion of purchased animals was expected to be associated with the prevalence, as this has been found in other studies (Wells and Wagner, 2000; Tiwari et al., 2009). But, herd size was not associated with changes in prevalence, perhaps because the purchase behavior, and not herd size, is an important factor for changes of the MAP prevalence.

The effect of the other management factors seemed somewhat uncertain, except for use of waste milk, which was shown to have limited effect on the transmission of MAP (Nielsen et al., 2008). The results on use of waste milk provided contradicting results, because use of waste milk from green cows only (considered best practice) resulted in greater prevalence of MAP-specific antibodies than did use of waste milk from yellow and green cows (considered second-best practice; Table 3). Furthermore, the majority of herds that did not use waste milk from red cows were distributed in relatively small groups when combined with the other significant risk factors. Therefore, use of waste milk from red cows was not represented among the 8 most common practices shown in Figure 1. Several potential risk factors yielded similar contradictory results in the univariable analyses (Table 2), but these findings were nonsignificant. The findings from the univariable analyses point to an interesting result: the hypothetical second-best practice resulted in faster prevalence decrease than the anticipated best practice in several instances. For example, the risk factor "calves removed within 2 h from …" yielded a lower estimate when calves were removed only from red cows, compared with removal from both red and yellow cows. This may suggest that farmers stating that removal within 2 h was done only for red cows were more honest about their actual practice, or more thorough when managing red cows, than farmers stating that they removed calves within 2 h from both red and yellow cows.

Several of the risk factors may be highly correlated. For example, a farmer addressing risk of transmission via colostrum may be more likely to also address transmission via waste milk. A variable combining waste milk and colostrum usage was evaluated, but this was abandoned because it was nonsignificant and difficult to interpret (data not shown). Generally, the effect of one risk factor may correlate to another and, therefore, we cannot decide in a univariable analysis which of the risk factors actually matters most. Furthermore, including several risk factors in a multivariable might introduce confounding effects between them; however, in our case the multivariable model was mostly developed to consider the additive effect of each risk factor on the slope for TIME. Our finding, that purchase pattern and red cows calving again are most important, does not appear counterintuitive to our biological understanding of the processes involved in transmission of MAP within and between herds.

Use of questionnaires at 1 time point to study retrospectively the effects of management practices has its disadvantages. Nondifferential misclassification is very likely to occur, resulting in lack of association or even reversal of the hypothetical association (Rothman and Greenland, 1998). Many management practices differ from animal to animal, and over time within a herd. For example, removal of calves from a high-risk dam within 2 h is less likely to occur when calving is at night than during the day, and new staff may change the management over time. We only asked about management practices at 1 time point. The large sample size may, to some extent, correct for this long-term, when more prevalence data have been collected. Self-reporting is inherently problematic and farmer responses can be divided into 3 broad categories: 1) accurate descriptions of management practices; 2) non-accurate descriptions of management practices, but the farmer does perform certain management practices that decrease MAP transmission; and 3) non-accurate descriptions, where the farmer attempts to provide the answer he believes is the most correct. All 3 types are likely present here. Given the distribution of answers in Tables 2 and 3, many farmers admit that they do not always follow best practice (e.g., calving area was not cleaned after calving in 582 of 1,081 herds; Table 2). Therefore, it might be justified to see the responses as stated practices, and some of these practices are associated with a significant decrease in prevalence of MAP-specific antibodies.

So far, the prevalence data cover the period from 0 to 4.25 yr after availability of the first test results. Many infected animals may only become antibody-positive after reaching the age of 4 yr due to the long incubation period. Therefore, some of the longer-term effects may only show within the next 5 to 10 yr, requiring that the prevalence is monitored longer and that the analyses are repeated when more data become available. We discarded many observations because the antibody test was replaced, and the models could not adequately take this into account, because of differences in (the partially unknown) misclassification bias provided by the 2 tests. This suggests that it would be beneficial to continue with the same test throughout the following period to avoid this effect or to adopt Bayesian methods that are more capable of addressing the issue of misclassification (Branscum et al., 2004). Although relatively few observations from each herd were available, significant evidence of the clustering within herd was observed (autocorrelation = 0.83).
Prevalence estimation and choice of model have been a challenge, because of the expected a time lag in the decrease in the prevalence. This time lag was expected because it would take some time before risk factors affecting transmission would be detectable, because animals are tested as cows but are generally presumed to be infected as calves. The data suggest that an immediate decrease in the prevalence occurred, probably because of a significant effect of the culling of red cows. These cows have a theoretical high effect on the infectious load in the herd, because antibody-positivity is a predictor of bacterial shedding of large amounts of MAP (Nielsen, 2008). Although a short-term effect in the decrease in prevalence can be attributed to culling of red cows, the long-term effect must be due to the removal of infectious animals, thereby decreasing transmission, rather than due to culling alone. If transmission is not broken, newly infected cows will continuously become test-positive, and culling alone is not expected to keep up with these new infections (Kudahl et al., 2007). Culling of red cows may have a greater effect because antibody-positivity may often precede large bacterial shedding of MAP (Nielsen, 2008), thereby being an early indicator of infectiousness, allowing for the farmer to respond to the test results before the cow becomes a risk to the herd.
To conclude, culling of repeated antibody-positive cows can be associated with a decrease in the prevalence of MAP-specific antibodies over a period of 4 yr; however, the proportion of purchased animals had more influence on changes in prevalence. Use of waste milk from repeated antibody-positive cows may also influence the prevalence of MAP-specific antibodies, but too few herds practiced this to make useful interpretations. No other management factors associated with a decrease in the prevalence of MAP infections was detected, but longer-term studies are required to elucidate these effects.

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