Age at Occurrence of *Mycobacterium avium* Subspecies *paratuberculosis* in Naturally Infected Dairy Cows

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ABSTRACT

Paratuberculosis is a chronic infection of ruminants and other species caused by *Mycobacterium avium* ssp. *paratuberculosis* (Map). Establishing test strategies for paratuberculosis will require insight into the temporal aspects of certainty with a given test. In this study, the age at which cows tested positive by ELISA and fecal culture (FC) was investigated by use of time-to-event analyses. The effects of herd, parity, and shedding group were evaluated at the age of test-positive ELISA and FC, respectively. Finally, the test frequency was investigated for the probability of cows being tested ELISA-positive. Milk and fecal samples were collected repeatedly over a 3-yr period from 1,776 Danish dairy cows from 8 herds. The milk samples were tested for the presence of antibodies by using an ELISA, and an FC test was used for detection of Map. Repeated ELISA testing detected 98 and 95% of cows classified as high and low shedders, respectively, suggesting that most infected cows develop antibodies. Among the high shedders, 50% were positive before 4.3 yr of age (quartiles 1 to 3: 3.4 to 5.7 yr of age). Repeated FC detected only 72% of the cows that were ELISA-positive, and 50% of the ELISA-positive cows were detected by FC at 7.6 yr of age. The age with the highest probability of testing positive was determined as the interval with the steepest slope in the survival probability plots. The highest probability of testing positive by ELISA was from 2.5 to 4.5 yr of age. The highest probability of testing positive by FC was from 2.5 to 5.5 yr of age. For both ELISA and FC, testing positive was highest in the first 300 d in milk. For cows younger than 4 yr of age, monthly testing with ELISA, compared with testing every 2 yr, could increase the probability of detecting cows with antibodies by 19%. In older cows, there were no apparent differences in the probability of testing positive by monthly sampling compared with sampling every second year. Therefore, for older animals the ef-

fect of more frequent sampling would be for early detection rather than to obtain additional information. Cows shedding high numbers of Map will produce antibodies, although not necessarily concomitantly with the shedding. These antibodies can be detected by ELISA with a test strategy that is different for younger and older cows. We suggest testing younger cows more frequently than older cows and that testing should be done prior to 350 d in milk.

Key words: antibody occurrence, bacterial shedding, paratuberculosis, test strategy

INTRODUCTION

Paratuberculosis in cattle is a chronic infection caused by *Mycobacterium avium* ssp. *paratuberculosis* (Map; Chiodini et al., 1984). Diagnosis is usually made by detection of Map by fecal culture (FC), or by detection of antibodies (indicating immune responses) by ELISA. Detection by FC requires that Map be shed in numbers high enough to elicit a positive culture, and ELISA requires that animals have antibodies (i.e., Map is seroconverted). The infection is initially controlled by a predominating T helper 1 (Th1) response, whereas loss of control occurs via a predominant Th2 response (Stabel, 2000). During the Th1 response, Map is shed in small numbers, which may be sufficient to elicit a positive FC. The pattern of bacterial shedding is not well characterized in naturally infected animals for the various phases of the infection. Experimental infections suggest that bacterial shedding decreases 10 to 14 mo after inoculation, but increases again later. Seroconversion is seen around 10 mo postinoculation (Lepper et al., 1989); however, experimental studies cannot be used as indicators of when bacterial shedding and seroconversion occur because of the variation in infective doses occurring during natural infections with Map. Studies using fixed dosages and known times of infection have resulted in great variation in the time to occurrence of FC-positivity and ELISA-positivity (Lepper et al., 1989). The temporal variation in pathogenetic events may be further enhanced if the size and number of dosages varies. Chiodini et al. (1984) reported that the immunological and infectious properties of the infection

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are not fully characterized, but stated that most cases occur between 3 and 5 yr of age.

The ability of a test to diagnose paratuberculosis will be affected by the age of an animal because of the chronic nature of the infection. The variation in age at seroconversion or bacterial shedding is a factor that can greatly affect the sensitivity of a test when determined in a cross-sectional study, and age-specific sensitivities of a test would have to be estimated. Therefore, it is important to know the age when seroconversion and bacterial shedding occur in infected animals if the test is expected to detect these 2 events. Test sensitivities in the range of 7% (McKenna et al., 2005) to 45% (Collins et al., 2005) were demonstrated in recent evaluations of a range of ELISA tests. Likely explanations for the low and variable sensitivities could be that antibodies are not present because of the distribution of stages of infection in the cross-sectional samples, or that the ELISA was unable to detect occurring antibodies. A report of sensitivities estimated, relative to stage of infection, a 15% sensitivity of ELISA in detecting low Map-shedding cows and an 87% sensitivity of the same ELISA in detecting clinical cases (Sweeney et al., 1995).

Jubb and Galvin (2000) described the mean age of detection of ELISA-positive cows as 5.6 yr, but with a range of 2 to 18 yr. Similarly, van Schaik et al. (2003) and Kalis et al. (1999) demonstrated that older animals are more likely to be positive by ELISA and FC, respectively. None of the studies included time-to-event analyses (also called survival analyses), which would be more appropriate to manage censored animals and skewed distributions of the time to an event. In survival analyses, the time until an event occurs is regarded as the outcome variable (Hosmer and Lemeshow, 1999). The event could be ELISA-positive and the time would be time to ELISA-positivity for an individual animal. Censored animals would not have an observed time to an event, because the event does not occur for these animals for different reasons (e.g., withdrawal or an insufficient period of time for the event to occur). Because time-to-event data are continuous, it would be obvious to perform an ANOVA or linear regression to evaluate the effect of risk factors on the time to an event. Moreover, the assumption of normally distributed residuals is often violated because the distribution of time to an event is often skewed.

Our primary objectives were to determine 1) the time from birth to test-positivity and 2) the DIM at test-positivity. Test-positivity was defined as a) the occurrence of antibodies, estimated by a milk ELISA response, and b) the occurrence of detectable bacterial shedding, estimated by a fecal sample response. Our secondary objectives were to determine the influence of

herd, parity, and shedding group on the time from birth to being test-positive (using the milk ELISA response and bacterial shedding, respectively). Finally, we wished to determine the effect of sampling frequency on test-positivity using the milk ELISA response.

MATERIALS AND METHODS

Herds and Animals

Samples were obtained from 8 Danish dairy herds from February 2000 to March 2003. Herd sizes ranged from 66 to 260 cows from October 1, 1999, to September 30, 2000. Herd milk production was between 5,922 and 10,060 kg of FCM per cow. Additional information regarding the herds is available in Nielsen and Toft (2006). Milk samples were collected from lactating cows 11 times/yr through the Danish milk recording scheme, and fecal samples were collected 4 times/yr from all cows present in the herd at the date of sampling.

Diagnostic information was collected from 1,885 cows. Information on dates of birth, dates of calving, and breed was obtained from the Danish Cattle Database. Cows ($n = 100$) without ELISA and FC test results were excluded, as were 9 Red Danish cows. The breed distribution of the remaining 1,776 cows was 1,260 Danish Holsteins, 407 Danish Jerseys, and 109 cross-breeds. Age was calculated as the difference between sampling date and date of birth. For some analyses, the cows were divided into parity groups. These groups were parities 1, 2, and ≥ 3 , corresponding to the grouping in Nielsen et al. (2002). The distribution of milk samples from these cows was as follows: minimum: 1 sample/cow; 25th percentile: 6 samples/cow; median: 11 samples/cow; 75th percentile: 18 samples/cow; maximum: 30 samples/cow. The distribution of fecal samples per cow was as follows: minimum: 1 sample/cow; 25th percentile: 3 samples/cow; median: 4 samples/cow; 75th percentile: 7 samples/cow; maximum: 13 samples/cow.

Diagnostic Procedures

Milk samples were analyzed for the presence of antibodies using an absorbed indirect milk ELISA, based on an *M. avium* antigen, as previously described (Nielsen, 2002). The resulting measure was a corrected optical density, which was obtained by subtracting the mean optical density of a set of negative controls from the sample optical density. An animal was defined as antibody-positive on the date the moving average of 2 consecutive measurements was greater than a corrected optical density of 0.3 and the minimum of these 2 measurements was greater than 0.1. This test interpretation should provide a minimum specificity ranging from 0.997 at 2 yr of age to 0.93 at 5 yr of age, assuming

that 8 negative FC tests obtained over a 2-yr period could be used as a reference for noninfected animals (Nielsen and Toft, 2006).

Fecal samples were cultured on Löwenstein–Jensen medium through July 2002 and on Herrold's egg yolk medium beginning in August 2002 (Nielsen et al., 2004). The latter procedure appeared more sensitive (Nielsen et al., 2004), although this was not observed in a prospective study (Nielsen and Toft, 2006). All positive isolates were confirmed for the presence of the IS900 insertion sequence by PCR. For the FC analysis, the date on which Map was detected was the date the cow was considered positive. To evaluate the antibody response, FC-positive cows were divided into 2 shedding groups: FC_{High} and FC_{Low}, where FC_{High} was culture-positive cows with high bacterial counts (>10 cfu/g) or many repeated FC-positive tests without any nonzero counts of bacteria in between. The remaining FC-positive cows were classified as FC_{Low}. These shedding groups were the same as those used in Toft et al. (2005). A thorough descriptive analysis of test responses is given in Nielsen and Toft (2006).

Statistical Analysis

The primary outcome variable was "age at test-positivity," defined as days from birth to either date of being tested positive (event) or the end of the study period (censored observation) for test-negative animals. Test-positivity was defined as antibody-positivity (ELISA) or shedding level (FC). The second outcome variable was "DIM at test-positivity," which was used to determine whether the test-positive animals were equally distributed over the lactation. Days in milk at test-positivity was defined as days from calving to either the date of being tested positive or the end of the lactation (censored observation) for test-negative animals. Risk factors were herd (1 to 8), shedding group (FC_{Neg}, FC_{Low}, FC_{High}), and parity (1, 2, and ≥3). For DIM at FC-positivity as the outcome, antibody status (positive, negative) was included as a risk factor.

First, the descriptive analysis for being test-positive stratified by the risk factors was computed by means of a frequency distribution for ELISA and FC, respectively. The distributions of age and DIM at being test-positive were computed by means of the median and quartiles (Q₁ and Q₃) for milk and fecal samples, respectively.

A survival analysis using a Cox proportional hazards regression model was used to evaluate the effect of risk factors on age at test-positivity and DIM at test-positivity. In a Cox proportional hazards regression model, no particular form is required for the survival times, and in particular, the baseline hazard function is un-

Table 1. Distribution of antibody-positive and antibody-negative cows stratified by shedding group, herd, and parity¹

Variable	Level	Negative		Positive	
		n	%	n	%
Total		1,174	66.1	602	33.8
Shedding group	FC _{Neg}	1,026	71.8	402	28.2
	FC _{Low}	86	42.4	117	56.9
	FC _{High}	62	42.8	83	57.2
Herd	1	94	69.1	42	30.9
	2	171	76.3	53	23.7
	3	167	61.9	103	38.1
	4	101	67.3	49	32.7
	5	120	57.1	90	42.9
	6	319	65.2	170	34.8
	7	83	61.9	51	38.1
	8	119	73.0	44	27.0
Parity	1	407	66.1	209	33.9
	2	346	62.2	210	37.8
	≥3	421	69.7	183	30.3

¹FC_{Neg} = Fecal culture-negative cows; FC_{High} = fecal culture-positive cows with high bacterial counts (>10 cfu/g) or many repeated fecal culture-positive tests without any nonzero counts of bacteria in between; FC_{Low} = remaining fecal culture-positive cows.

specified. The baseline hazard function is an arbitrary function of time and does not have to be specified explicitly. The Cox proportional hazards regression model is a semiparametric analysis. The single assumption is proportional hazards because the ratio of the hazards for any 2 animals is assumed to be constant over time.

In the survival analyses, the outcome variables were age at test-positivity and DIM at test-positivity. Separate analyses were made for test-positivity, defined by using ELISA on milk samples and FC in fecal samples, respectively. The significance level of the risk factors was computed using the likelihood ratio statistic and a χ^2 distribution (Collett, 2003). All risk factors were initially included in the model. Interactions between risk factors were not evaluated. Backward elimination of nonsignificant ($P > 0.05$) risk factors was used. The hazards ratio and the 95% confidence intervals for significant risk factors were calculated.

The assumption about proportional hazard functions when using Cox proportional hazards models was evaluated by visual inspection of $\log\{-\log[S(t)]\}$ vs. $\log[S(t)]$ plots, where $S(t)$ is the survival function.

RESULTS

The descriptive statistics with distribution of antibody status and the distribution stratified by shedding group, herd, and parity are shown in Table 1. The distributions of the outcomes, DIM and age, are given in Table 2, divided into antibody-positive and antibody-negative cows. Where the FC was assessed, descriptive statistics of the data are provided in Tables 3 and 4.

Table 2. Distribution by age and DIM for antibody-positive and antibody-negative cows

Variable	Negative			Positive		
	n	Median	Q ₁ to Q ₃ ¹	n	Median	Q ₁ to Q ₃
Age, yr	1,174	4.1	3.3 to 5.3	602	3.9	3.2 to 4.9
DIM	1,174	209	103 to 329	602	217	125 to 301

¹Q₁ = First quartile; Q₃ = third quartile.

The resulting multivariable Cox proportional hazards models for age and DIM at detection of antibodies are presented in Table 5. The risk factor “herd” was not significant in either model, whereas “shedding group” was significant in both models. There was no difference between FC_{High} and FC_{Low} in either of the models. Cows from the FC_{Low} group were 2.1 times more likely to test positive with ELISA than cows that were never FC-positive, and cows from the FC_{High} group were 2.9 times more likely to be ELISA-positive, as indicated by the hazards ratio.

The resulting survival probability functions of age and DIM are shown in Figures 1 and 2, respectively. The median age for being ELISA-positive was 6.1 yr (Q₁ to Q₃: 4.2 to 10.9). For the FC_{High} group, the median age was 4.3 yr (Q₁ to Q₃: 3.4 to 5.7). At 11.7 yr, 98% (95% confidence interval: 0.91 to 1.0) of the FC_{High} cows had become antibody-positive (Figure 1). Thus, 98% for the FC_{High} cows developed antibodies. For the FC_{Low} cows, 95% developed antibodies.

In the range of 0 to 350 DIM, the slopes of the survival probability curves for all groups were almost linear (Figure 2), suggesting that the risk of a cow becoming test-positive was equally distributed in this period. After 350 DIM, the slopes were not as steep, indicating that the probability of testing positive decreased late in lactation.

Table 3. Distribution of fecal culture-positive and culture-negative cows overall and stratified by ELISA group, herd, and parity

Variable	Level	Negative		Positive	
		n	%	n	%
Total		1,428	80.4	348	19.6
ELISA	Negative	1,026	87.4	148	12.6
	Positive	402	66.8	200	33.2
Herd	1	107	78.7	29	21.3
	2	211	94.2	13	5.8
	3	249	92.2	21	7.8
	4	130	86.7	20	13.3
	5	145	69.0	65	31.0
	6	374	76.5	115	23.5
	7	70	52.2	64	47.8
	8	142	87.1	21	12.9
Parity	1	404	72.7	152	27.3
	2	431	81.3	99	18.7
	≥3	593	85.9	97	14.1

Table 6 gives the results for models based on FC. Herd and parity group were not significant. Only the risk factor “antibody-positivity” was significant in both analyses. Antibody-positive cows were 2.3 times more likely to shed detectable amounts of Map with aging, relative to antibody-negative cows. The survival probability functions of age and DIM for FC are shown in Figures 3 and 4, respectively. The median for being FC-positive was at 11.2 yr of age (Q₁ to Q₃: 5.6 to >11.3). Of the antibody-positive cows, 50% had become FC-positive at 7.6 yr of age (Q₁ to Q₃: 4.3 to >11.3; Figure 3). For ELISA-positive as well as ELISA-negative cows, the probability of being FC-positive was similar from 0 to 300 DIM (Figure 4), whereas few cows tested positive later in lactation.

For repeated ELISA tests, the effect of the interval between sampling on the probability of detecting cows shedding Map is illustrated in Figure 5 by depicting 1 minus the survivorship function. Survivor functions from 4 to 24 mo were almost identical for the FC-positive cows. Hence, only the strategy with all samples included (~9 to 10 for a 305-d lactation) and the strategies with 2, 3, 4, 6, and 24 mo between samplings are shown. At yr 3 of age, the drop in the probability of detecting FC-positive cows with ELISA using the 24-mo strategy rather than the “all” strategy was 0.06. At yr 3.5 of age, the drop was 0.13; at yr 4 of age, the drop was 0.18; at yr 5 of age, the drop was 0.18; and at yr 6 of age, the drop was 0.19 (Figure 5), suggesting that a higher sampling frequency is of greater value until yr 4 of age and not later.

Visual inspection of the log{−log[S(t)]} vs. log[S(t)] plots showed parallel curves when stratified by the different risk factors, indicating that the assumption regarding proportional hazards was fulfilled.

Table 4. Distribution of age and DIM for fecal culture-positive and culture-negative cows

Variable	Negative			Positive		
	n	Median	Q ₁ to Q ₃ ¹	n	Median	Q ₁ to Q ₃
Age, yr	1,428	4.3	3.4 to 5.6	356	3.6	2.9 to 4.7
DIM	1,428	224	98 to 324	348	179	78 to 287

¹Q₁ = first quartile; Q₃ = third quartile.

Table 5. Resulting multivariable Cox proportional hazards models of risk factors associated with age and DIM when cows tested positive based on antibodies in milk samples¹

Variable	Level	Estimate	SE	HR	95% CI	Overall <i>P</i>
Age						
Shedding group	FC _{Neg}	0	—	1	—	<0.001
	FC _{Low}	0.75	0.11	2.11	1.71 to 2.59	
	FC _{High}	1.06	0.12	2.88	2.27 to 3.65	
DIM						
Shedding group	FC _{Neg}	0	—	1	—	<0.001
	FC _{Low}	0.78	0.11	2.18	1.77 to 2.68	
	FC _{High}	1.09	0.12	2.98	2.35 to 3.78	
Parity	1	0	—	1	—	<0.001
	2	0.35	0.09	1.42	1.19 to 1.70	
	≥3	0.31	0.12	1.37	1.08 to 1.73	

¹FC_{High} = Fecal culture-positive cows with high bacterial counts (>10 cfu/g) or many repeated fecal culture-positive tests without any nonzero counts of bacteria in between. FC_{Low} = remaining fecal culture-positive cows; FC_{Neg} = fecal culture-negative cows; HR = hazards ratio; CI = confidence interval.

Figure 1. Survival probability plot of age at the occurrence of antibodies in cows: average; cows without detected shedding of *Mycobacterium avium* ssp. *paratuberculosis* (Never FC+, where FC refers to fecal culture); cows with a low level of shedding (FC_{Low}); and cows with high levels of shedding (FC_{High}). For each FC group, vertical lines are drawn at the age at which 50% of the group tested positive. For each survivor function, the 95% confidence interval is shown by shaded areas around the graph.

Figure 2. Survival probability plot of DIM at the occurrence of antibodies against *Mycobacterium avium* ssp. *paratuberculosis* (Map) in cows by parity and by shedding group. Survival functions in black show cows with high levels of Map (FC_{High}, where FC refers to fecal culture); survival functions in dark gray show cows with low levels of Map (FC_{Low}); and survival functions in light gray show cows whose FC were always negative (FC_{Neg}).

DISCUSSION

A frequent statement made in veterinary medicine textbooks on cattle is that paratuberculosis most frequently occurs in cows between 3 and 5 yr of age (Chiodini et al., 1984; Radostits et al., 2000), which would be expected because most cows are of this age. For example, 65% of Danish dairy cows ($n = 671,584$) were 3 to 5 yr old on December 31, 2005, based on information

from the Danish Central Herd Register. However, time-to-event analyses providing evidence for the statement have not been performed. This statement is partly concordant with the results of the present study: From 2.5 to 4.5 yr of age, the survivorship (i.e., survival probability) function of ELISA-positivity was steepest (for FC-positive cows). For ELISA-positive cows, the survivorship function of FC-positivity was steepest from 2.5 to 5.5 yr of age, thereby indicating that the speed of

Table 6. Multivariable Cox proportional hazards models of risk factors associated with age and DIM when cows tested positive by fecal culture¹

Variable	Level	Estimate	SE	HR	95% CI	Overall <i>P</i>
Age ELISA	Negative	0	—	1	—	<0.001
	Positive	0.83	0.11	2.28	1.84-2.82	
DIM ELISA	Negative	0	—	1	—	<0.001
	Positive	1.06	0.11	2.87	2.32-3.55	

¹HR = Hazard ratio; CI = confidence interval.

Figure 3. Survival probability plot of age at detection of *Mycobacterium avium* ssp. *paratuberculosis* in cows: average; cows testing ELISA-positive; and cows testing ELISA-negative. For each group, vertical lines are drawn at the age at which 50% of the group tested positive. For each survivor function, the 95% pointwise confidence interval is shown by shaded areas around the curve.

conversion to positivity was highest at this age. Moreover, 40 to 50% of the animals became test-positive after 5 yr of age. Therefore, although many animals do not become test-positive prior to reaching old age, establishing the most cost-effective test strategies requires including the expected test responses.

The immediate conclusion of the present study is that cows are shedding detectable amounts of Map and that they develop antibodies at some point in time (Figure 1). The test sensitivity is higher in the first 300 DIM (Figure 2). One-quarter of the cows with antibodies do not shed detectable amounts of Map (Figure 3). The sensitivity of FC is slightly higher in the beginning of lactation (<300 d; Figure 4). A monthly testing frequency during lactation can increase the probability of detecting Map-shedding cows by ELISA by approximately 18 to 19 percentage points, an increase primar-

ily obtained through high-frequency testing prior to 4 yr of age (Figure 5). This means that with monthly testing of cows <4 yr of age, 42% of cows testing FC-positive sometime during their lifetime would be detected, whereas with twice annual testing, only 24% of the same FC-positive cows would be detected. For older cows, this difference did not exist. For the older cows, the advantage of frequent testing would be earlier detection.

The interpretation of these results may still be a little confusing, primarily because no test has yet been accepted as the gold standard; that is, whether ELISA or FC should be used as the reference has not been determined. Neither test has perfect specificity in infected herds because passive uptake of Map without subsequent infection may lead to false-positive FC, and immune reactions to infections with other mycobacteria

Figure 4. Survival probability plot of DIM at detection of *Mycobacterium avium* ssp. *paratuberculosis* in cows: average and by ELISA group. For each survivor function, the 95% confidence interval is shown by shaded areas around the curve.

may lead to false-positive ELISA. Thus, neither test should be used as a reference for freedom from infection. The specificity of the ELISA was estimated at 0.99 for cows 2 yr of age and at 0.93 for cows 5 yr of age. The specificity of FC was estimated at between 0.96 and 0.99 during the same age span (Nielsen and Toft, 2006). As a result, a number of cows in the present study were misclassified. With the higher test frequency of ELISA, the serial specificity of ELISA is lower than the serial specificity of FC. Therefore, more cows classified as ELISA-positive in the analysis of the FC response would be misclassified than cows classified FC-positive in the analysis of the ELISA response.

Our findings suggest that cows shedding measurable amounts of bacteria will develop antibodies (Figure 1), but FC cannot detect all cows with antibodies (Figure 3). The utility of the ELISA test in detecting cows shedding high numbers of bacteria has been demonstrated here. Which of the 2 events occurs first is highly variable and is required to determine whether the use of this utility should be operational. A thorough characterization of these aspects is beyond the objectives of this study.

Because antibody production and bacterial shedding occur over the entire age period studied, the diagnostic sensitivity at a given age cannot be very high, which is consistent with the low sensitivity estimates reported by McKenna et al. (2005). Assuming infection in calf-hood, the probability of detecting the shedding of detectable numbers of Map in cows 4 yr of age or older is only 40 to 50% with repeated testing (Figure 1). Age is an important factor in establishing test strategies. The time of testing during lactation appears less important, although ELISA testing later than 350 DIM appears to reduce the sensitivity to some extent (Figure 3). The drop in the probability of testing FC-positive late in lactation is less pronounced (Figure 4). More important is the testing frequency with ELISA, which surprisingly has a primary effect until 4 yr of age. This means that frequent testing of young cows can provide more information than frequent testing of older cows. In young cows, the specificity of the ELISA is highest (Nielsen and Toft, 2006); thus, the drop in specificity would have less effect on the serial specificity than later in life. Monthly sampling of young cows could increase the sensitivity of ELISA and would be advisable, although

Figure 5. Effect of sampling interval on the probability of testing positive for *Mycobacterium avium* ssp. *paratuberculosis* (Map; 1 – survivor probability function) in the milk ELISA with repeated sampling. Sampling frequency varied from 2 to 24 mo (see legend). The top 6 lines are from cows testing Map-positive by fecal culture, whereas the lower 6 lines in the graph are from cows testing Map-negative.

a cost–benefit analysis to determine the optimal frequency of testing should be performed. The price of an ELISA relative to FC provides additional motivation for the use of this test. In Denmark, the cost of an ELISA, including sampling, is currently DKK 23 (roughly \$4.00). The cost of an FC, including sampling, is approximately 185 DKK (roughly \$32.00), which is equal to 8 ELISA tests performed at the cost of one FC, and with a huge gain in information. For cows older than 4 yr, less frequent sampling is needed, resulting in additional savings.

This study has some strengths and weaknesses because of its observational nature. The primary strength is that the cows in the study were naturally infected, and selection bias should not be a major factor in assessing the occurrence of antibodies and the shedding

of Map. The primary weaknesses are that cows provided a variable number of samples, and followup analyses on slaughterhouse material were not possible, because the cows were sent to multiple slaughter facilities, a factor beyond our control. Postmortem studies on the cows could have provided additional information on their true infection status.

CONCLUSIONS

Almost all cows shedding high numbers of Map also showed antibodies within their lifetime. Cows became test-positive by ELISA and FC during the entire life span, from 2 to 11 yr of age, although the risk of testing positive by ELISA was greatest from 2.5 to 4.5 yr of age. Cows should preferably be tested in the first 350

DIM. Monthly testing with ELISA could increase the sensitivity substantially for cows <4 yr of age, but no gain was apparent from more frequent testing of older cows, particularly among cows shedding detectable amounts of bacteria.

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