Association Between the Presence of Antibodies to *Mycobacterium avium* subspecies *paratuberculosis* and Somatic Cell Count

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ABSTRACT

Somatic cell counts (SCC) in bulk tank milk delivered for human consumption are one of the indicators of milk quality and are used for milk pricing. Consequently, milk from cows with high SCC is frequently used by farmers for feeding of calves to lower the SCC in bulk tank milk. Young calves are more susceptible to *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and may acquire the infection early in life through ingestion of MAP-contaminated milk. The occurrence of MAP antibodies can be an indicator of MAP shedding. Because MAP can be shed in milk from infected cows, and antibodies to MAP can be an indicator of the infectious status, an association between antibodies to MAP and high SCC can result in high-SCC milk being at risk of containing MAP. Feeding milk containing high SCC to susceptible calves may result in MAP infections. Somatic cell counts and MAP antibodies in milk were measured repeatedly in 7,251 cows from 26 Danish dairy herds to investigate the association between the occurrence of MAP antibodies and high SCC. The results of robust regression showed a log-linear relationship between the age at first positive ELISA and the age at first high SCC sample ($R^2 = 0.51$). Of the 1,733 cows positive for MAP antibodies and with high SCC, high SCC was detected prior to MAP antibodies in 46% of the cows. Still, in 40% of the cows, MAP antibodies were detected before a high SCC. Therefore, the findings do not point to a causal relationship between high SCC and antibodies to MAP, but suggest a strong association and highlight a potentially increased risk of MAP transmission when milk with high SCC is fed to calves.

Key words: paratuberculosis, robust regression, somatic cell count

INTRODUCTION

Paratuberculosis is a chronic enteric infection of exotic and domestic ruminants, which is included in the World Organisation for Animal Health (OIE) List B diseases and caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP; Chiodini et al., 1984). *Mycobacterium avium* ssp. *paratuberculosis* affects the cattle industry around the world (Kennedy and Benedictus, 2001). Because of the chronic nature and the large percentage of subclinical infections (Whitlock and Buergelt, 1996), MAP infections are difficult to control. In Denmark, a study from 1999 indicated a prevalence of 80 to 85% in dairy herds (S. S. Nielsen, unpublished data) and a within-herd prevalence of 33% (Nielsen et al., 2007b). The primary route of transmission is via oral ingestion of excreted MAP. Shedding of MAP in feces, milk, and colostrum from infected dairy cattle has been described (Sweeney, 1996), although fecal contamination of collected milk should also be considered.

Younger animals are more susceptible to infection with MAP (Doyle, 1953), although clinical signs generally do not appear until adulthood or may never develop (Chiodini, 1996). During the incubation period, asymptomatic animals can shed MAP into the environment, resulting in further transmission of infection in the herd. Therefore, for each clinically diseased animal detected by the producer, there might be 15 to 20 animals infected within the herd (Whitlock and Buergelt, 1996). For an efficient MAP control strategy, repeat testing and establishment of management practices for the protection of susceptible calves are needed to break transmission routes (Groenendaal et al., 2002).

An increased susceptibility to other infections, such as mastitis, after MAP infections has been suggested (Merkal et al., 1975). Cows can become infected with mastitis pathogens and develop subclinical or clinical mastitis, which depends on several factors, such as management practices, environmental factors, and the condition of the immune system (Smith et al., 1985). Somatic cell count is a measure of the number of leukocytes and epithelial cells per milliliter of milk. In-

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creased SCC is indicative of mammary gland inflammation, and if associated with isolation of mastitis pathogens, indicates mastitis (Harmon, 1994). Somatic cell count is used for milk pricing as one of the indicators of milk quality and is a widely accepted criterion for representing the udder health status of a dairy herd.

A legal limit of 400,000 cells/mL was established by the European Union as the maximum SCC in bulk milk collected at the farm (Council Directive 92/46/EEC, European Economic Community, 1992), although various quality programs reward farmers additionally for lower SCC in bulk milk. Milk from high-SCC individuals increases bulk tank SCC results. Therefore, this price differentiation may lead to farmers withholding milk from cows with high SCC and feeding it to calves.

The presence of antibodies to MAP is correlated with shedding of MAP (Nielsen and Ersbøll, 2006; Nielsen and Toft, 2006). Because the ingestion of contaminated milk or colostrum can be a primary route for calf infection (Taylor et al., 1981), showing an association between a high SCC and the occurrence of antibodies to MAP can be crucial for effective control in dairy herds. During the course of a MAP infection, the immune response varies, first presenting a cell-mediated immune response in early subclinical stages and later a humoral immune response with production of antibodies (Stabel, 2000). Kreeger and Snider (1992) described a reduced immune system cell response as a consequence of chronic MAP infection, which may be the basis for the increased risk of secondary diseases. If an association between MAP infection and the occurrence of mammary infections is present, evaluation of the time between the occurrence of MAP antibodies and a high SCC may provide evidence of the underlying immune mechanisms. At least 3 different hypotheses of this interrelationship can be established: 1) progression of a MAP infection may lead to progression of mammary infections, resulting in a high SCC; 2) mastitis may be the primary cause for reduced immune competence, leading to the progression of a MAP infection and the occurrence of antibodies; and 3) an unknown factor unrelated to the MAP infection and mastitis may lead to progression of both MAP and mammary infections.

McNab et al. (1991) showed an association between MAP (defined by a lipoarabinomannan ELISA-positive result) and higher SCC. Nordlund et al. (1996) and Hendrick et al. (2005) found no significant differences between SCC in MAP-positive and MAP-negative cows. Nonetheless, all these studies were cross-sectional, and if there is a time lag between the 2 events, an association may be detected only in a longitudinal study. The objective of the present study was to determine and characterize the association between occurrence of

MAP antibodies and high SCC in dairy cattle in a longitudinal study with follow-up.

MATERIALS AND METHODS

Data Sources

The sample population consisted of all cows that were present in 8 Danish dairy farms from December 1999 to March 2003 (study period 1) and cows present in 19 dairy herds from August 2003 to November 2006 (study period 2). One herd was included in both study periods. In study period 1, SCC and ELISA testing were performed 11 times per year in each herd. In study period 2, SCC and ELISA testing were performed 4 times per year in each herd.

Data from repeated ELISA sampling, milk yield (kg), SCC records, and DIM were obtained through the Danish milk recording system. Information on calving identification and herd number, culling, parity, breed, and date of birth was extracted from the Danish Cattle Database. Table 1 summarizes information regarding age at first sampling for ELISA and SCC and the time at risk in each herd. The time at risk by herd was expressed as the sum of individual animal time units (cow years) from the beginning of the study to withdrawal or study end. *Mycobacterium avium* ssp. *paratuberculosis* was isolated on at least one occasion in all herds but one (herd 3), yet the antibody profile indicated MAP presence.

Of the 7,254 dairy cows sampled, 3 were excluded from the study because of missing breed information. Five different breeds were present and were distributed as follows: 1.7% Red Danish, 67.1% Danish Holstein (Black and White), 22.3% Danish Holstein (Red and White), 4.4% Danish Jersey, and 4.5% crossbreed.

Sampling was carried out longitudinally as a cross-sectional survey with follow-up. Therefore, the number of cows across the study periods, and consequently the number of samples per cow, varied as follows: ELISA: minimum = 1, median = 5, and maximum = 40; SCC: minimum = 1, median = 6, and maximum = 45.

Laboratory Analyses

Somatic cell counts of milk samples were determined according to the International Dairy Federation/International Organization for Standardization standard 148A (International Dairy Federation, 1995) by using a Fossomatic 5000 instrument (Foss, Hillerød, Denmark).

Milk samples were tested for antibodies to MAP by using an adapted ELISA based on a commercially available antigen (Allied Monitor, Fayette, MO), whose performance was reported previously (Nielsen and Toft,

Table 1. Characteristics of 26 Danish dairy herds included in the analysis in study periods 1 and 2¹

Herd	Time at risk, cow yr ²	Age distribution at first sampling					
		ELISA			SCC		
		Minimum	Median	Maximum	Minimum	Median	Maximum
1	189.4	1.8	2.5	9.1	1.9	2.4	9.1
2	726.0	1.7	2.4	10.8	1.8	2.4	10.8
3	414.0	1.9	2.6	8.1	1.8	2.6	8.1
4	212.9	2.0	2.6	11.0	2.0	2.6	11.0
5	320.5	1.9	2.7	12.3	1.9	2.7	12.3
6	666.5	2.0	2.6	13.0	2.0	2.6	13.0
7	186.7	1.3	2.6	11.0	1.9	2.5	11.0
8	252.7	2.1	2.8	8.1	2.1	2.8	8.1
9	571.1	1.9	2.4	8.8	1.9	2.5	9.0
10	618.8	1.9	2.8	10.9	1.8	2.8	10.9
11	325.7	2.1	2.7	11.7	2.0	2.6	11.3
12	265.3	2.0	2.9	8.9	2.1	2.9	8.7
13	347.4	2.0	2.6	10.0	2.0	2.7	10.2
14	336.1	2.0	2.6	9.4	2.0	2.5	9.4
15	168.3	1.8	2.8	9.0	2.0	2.8	9.0
16	354.3	1.9	2.7	8.6	1.8	2.6	8.7
17	70.0	1.8	3.1	9.4	1.9	3.0	9.4
18	293.6	2.1	2.5	9.2	1.8	2.5	9.0
19	129.7	1.9	3.6	9.1	2.1	3.5	8.7
20	570.2	1.5	2.9	11.3	1.7	2.9	11.3
21	277.5	1.9	2.5	13.6	1.5	2.5	13.6
22	303.0	1.7	2.4	8.1	1.7	2.4	8.1
23	347.9	2.0	2.8	7.7	2.0	2.8	7.3
24	168.4	2.0	2.6	8.1	2.0	2.6	8.6
25	236.3	1.9	2.7	11.0	1.9	2.9	11.2
26	170.7	2.0	2.7	7.9	1.9	2.7	7.7

¹Herds 1 to 8 correspond to study period 1. The remaining herds were in study period 2. Herd number 2 was present in both study periods.

²The time at risk by herd is expressed as the sum of individual animal time units (cow years) from beginning the study to withdrawal or study end.

2006). Each sample was tested in duplicate. Polysorb microtiter plates (Nunc, Roskilde, Denmark) were coated with the antigen (2 µg/mL in 0.1 M carbonate buffer, pH 9.6) and left for 3 d at 5°C. The samples were diluted 1:2 in *Mycobacterium phlei* (Allied Monitor) to absorb possible cross-reacting antibodies to non-*Mycobacterium avium* strains. The absorbed samples were left overnight at 5°C and washed 5 times in PBS (pH 6.8) with 0.05% Tween₂₀ (PBST₂₀). After adding the samples to the coated plates in duplicate (100 µL/well), they were incubated at 5°C overnight. Two sets of positive and negative controls were included on each plate. After 5 repeated washings with PBST₂₀, 100 µL of a 1:2,000 dilution of peroxidase-labeled goat antbovine IgG (H + L; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. The plates were incubated at 20°C and washed.

A solution of ortho-phenylene diamine (Kem-En-Tec, Copenhagen, Denmark) was diluted in citrate buffer (pH 5.0) to a concentration of 300 µg/mL. One hundred milliliters of this solution was supplemented with 80 µL of H₂O₂ and added to each well. The chemical reaction was stopped by adding 100 µL of 0.5 M H₂SO₄ to

each well. Optical density (OD) values were obtained by using an ELISA reader (492 nm) and a 620-nm filter as reference. For each sample, a corrected OD (ODc) was calculated by subtracting the mean OD value of negative controls from the mean OD value of a test sample. The ODc were used for statistical analysis.

Statistical Methods

Descriptive Statistics. The continuous SCC was dichotomized into high and low by using a cutoff of 300,000 cells/mL. An animal with at least 1 ODc >0.3 was defined as ELISA positive, based on the cutoff used in the Danish Paratuberculosis program (Nielsen et al., 2007a). All cows were categorized into parity groups (1, 2, and ≥3) and lactation stages (0 to 100, 101 to 200, and >200 DIM).

Descriptive statistics were carried out, providing the distributions of ELISA and SCC groups stratified by herd, parity group, and lactation stage, respectively. Age at first sampling, high SCC, and ELISA positivity were computed by herd, and the distributions of age at first positive detection were calculated.

Association Between High SCC and ELISA-Positive Results. Fisher's exact test was used to test the association between the ELISA-positive or ELISA-negative groups and the high or low SCC groups. The results were statistically compared by herd and breed.

Robust Regression Analysis. Robust regression analysis was used to assess the association between the age at first ELISA-positive sample and age at first high-SCC sample. Robust regression is a method that is more resistant to the presence of outliers than linear regression (Staudte and Sheather, 1990). The term outlier refers to data that appear unusual relative to the majority of the sample and may represent extraordinary (or unlikely) values of the dependent variable or data that are a result of errors in data collection. Robust regression can protect from high leverage points in the data (i.e., extreme values of the independent variable). Model fitting and parameter estimation were performed by using MM-estimation (Yohai, 1987) in the SAS procedure ROBUSTREG (Chen, 2002). The MM-estimation is a 3-step process in which the initial estimates are calculated by using least trimmed squares (which adjusts for outliers in the dependent variable) and subsequently adjusts to also account for outliers in the independent variable.

The general robust regression model was:

$$\log(\text{age at first positive ELISA}) = \alpha + \beta \log(\text{age at first high SCC}) + \text{breed} + \varepsilon,$$

where $\log(\text{age at first positive ELISA})$ is the log-transformed value of age at the first positive ELISA; α is the intercept; β is the slope for $\log(\text{age at first high SCC})$; $\log(\text{age at first high SCC})$ is the log-transformed value of age at first high SCC; breed is the fixed effect of breed on the $\log(\text{age at first positive ELISA})$; and ε is the random error. The log transformation was applied to improve the assumption of normally distributed observations.

Plots of residuals and predicted values were carried out to evaluate heteroscedasticity and the normal distribution of residuals. Residuals were tested for normality by using the Kolmogorov-Smirnov test. Leverage and outlier points were identified.

Additional Exploratory Analysis. Additional exploratory analysis was used to further explore patterns in the data, specifically the relationship between ELISA and SCC results and age.

The distribution of the 4 outcomes (ELISA positive/high SCC; ELISA positive/low SCC; ELISA negative/high SCC; and ELISA negative/low SCC) was evaluated by plotting the predicted values vs. age. The SAS procedure GAM was used to visualize the pattern among the

Table 2. Distribution of ELISA test results of Danish dairy herds stratified by herd, parity, lactation stage, and SCC group

Variable	Negative		Positive	
	n	%	n	%
Total SCC	4,631	64	2,620	36
Low	2,509	35	887	12
High	2,122	29	1,733	24
Herd				
1	96	62	59	38
2	334	73	122	27
3	140	47	161	53
4	78	47	87	53
5	146	61	95	39
6	293	50	293	50
7	57	39	88	61
8	111	60	74	40
9	231	53	208	47
10	346	76	110	24
11	213	74	74	26
12	122	61	77	39
13	180	68	83	32
14	180	67	89	33
15	102	61	65	39
16	274	72	104	28
17	120	84	23	16
18	177	73	65	27
19	125	65	66	35
20	505	71	202	29
21	112	57	85	43
22	97	43	127	57
23	207	74	72	26
24	93	66	48	34
25	134	64	77	36
26	158	71	66	29
Parity ¹				
1	1,923	78	540	22
2	1,227	58	880	42
≥3	1,386	55	1,149	45
Lactation stage, ¹ DIM				
0 to 100	1,196	63	710	37
101 to 200	1,053	67	508	33
>200	2,287	63	1,351	37

¹No information on parity and lactation stage was available for 146 cows.

variables, representing the data in a smoothed format. A generalized additive model was fitted by using a B-spline smoother with 3 degrees of freedom. All of the above analyses were performed in the software SAS 9.1.3 (SAS Inst. Inc., Cary, NC).

RESULTS

The distributions of observations used in the analysis by ELISA group (positive or negative) cross-tabulated by SCC group, herd, parity, and lactation stage group are given in Table 2. The distribution of the high-SCC and low-SCC cows stratified by ELISA group, herd, parity, and lactation stage is given in Table 3. The number of positive animals increased from parity group 1 to ≥3 for both SCC and ELISA. The distributions of

Table 3. Distribution of SCC test results of Danish dairy herds stratified by herd, parity, lactation stage, and ELISA group

Variable	Low		High	
	n	%	n	%
Total	3,396	47	3,855	53
ELISA				
Negative	2,509	35	2,122	29
Positive	887	12	1,733	24
Herd				
1	44	28	111	72
2	230	51	225	49
3	85	28	216	72
4	56	34	108	66
5	104	43	136	57
6	189	32	397	68
7	19	13	125	87
8	57	31	128	69
9	222	51	215	49
10	207	46	248	54
11	144	50	143	50
12	102	51	97	49
13	135	52	124	47
14	128	48	141	52
15	83	50	84	50
16	219	58	158	42
17	76	53	67	47
18	163	67	79	33
19	84	44	107	56
20	378	54	327	46
21	115	58	82	42
22	68	30	156	70
23	140	50	138	50
24	81	58	59	42
25	115	56	91	44
26	129	58	93	42
Parity ¹				
1	1,728	24	905	13
2	880	13	1,128	16
≥3	700	10	1,720	24
Lactation stage, ¹ DIM				
0 to 100	983	14	1,032	15
101 to 200	807	11	827	12
>200	1,518	21	1,894	27

¹No information on parity and lactation stage was available for 190 cows.

SCC values were first quartile (Q1): 59,000 cells/mL; median (Q2): 131,000 cells/mL; third quartile (Q3): 318,000 cells/mL; and maximum (Q4): 9999,000 cells/mL (detection limit).

The distributions of age at first ELISA-positive sample and age at first high-SCC sample for each herd are illustrated by box plots in Figure 1A and 1B, respectively. Outliers for each herd are shown. For each of the 26 herds, a similar age distribution was found for both the ELISA-positive and high-SCC first sample.

Of the 7,251 animals studied, 2,620 (36.1%) were positive by ELISA at least once. Among the ELISA-positive animals, 1,733 (66.2%) had at least 1 SCC record above the selected cutoff value. In a Fisher's exact test, a significant association was found between high SCC and ELISA positivity ($P < 0.001$). The relative risk of

high SCC for an ELISA-positive animal was 1.60 compared with ELISA-negative animals. A significant association was found when performing the analysis by breed and herd.

The "age at first positive ELISA" plotted against "age at first high SCC" is shown in Figure 2 (2,509 animals were found both negative to ELISA and with low SCC and consequently were not plotted). No cows under 2 yr of age were either ELISA positive or had high SCC, although the average age at first sampling was 1.9 yr. The linear appearance of the graph supports the view that a MAP-positive classification is strongly associated with a high SCC. This association was further examined by robust regression.

Preliminary separate analyses for each of the 2 study periods indicated that there were no differences in the results of the regression analyses; therefore, the data were combined into one data set. The association between "age at first positive ELISA" and "age at first high SCC" obtained by robust regression was:

$$\begin{aligned} \log(\text{age at first positive ELISA}) &= 0.339 \\ &+ 0.786 \times \log(\text{age at first high SCC}). \end{aligned}$$

The results imply that when cows have high SCC and are ELISA positive, the former is expected to occur first, but the expected time between the 2 events increases with an increase in age at first high SCC.

Breed was not significant ($P = 0.89$) and was excluded from the final model. Results from fitting the robust regression model via MM-estimation are summarized in Table 4. The coefficient of determination (R^2) was 0.51. For comparison, ordinary least squares regression yielded a $R^2 = 0.72$. After performing the log-log age transformation, the distribution of residuals was found suitable for the model.

The time of occurrence of MAP antibodies and SCC above the specified cutoff values for animals that both tested positive in ELISA and had high SCC were distributed as follows: 46% had high SCC prior to positive ELISA, 40% were first ELISA positive, and 14% tested positive in ELISA simultaneously with a high SCC. Plots of the predictions of SCC and ODC for the 4 considered outcomes (++; +−; −+; —) by the independent variable age are shown in Figure 3.

DISCUSSION

The results show a strong association between the age of occurrence of high SCC and the age of occurrence of MAP antibodies. Nearly 59% of the animals tested across the study periods revealed a consistent association between ELISA and SCC (either positive/high or negative/low). Robust regression computed a slope sig-

Figure 1. Comparative box plots showing the age distribution of Danish dairy cows by herd at first sampling of (A) positive ELISA and (B) high SCC.

Figure 2. Log relationship between the age at first positive ELISA and high SCC distribution of all animals included in the sample. Animals that were only positive to ELISA are plotted on the vertical axis ($x \approx 3$), and those with high SCC are plotted on the horizontal axis ($y \approx 3$). Breeds: BW = Danish Holstein (Black and White); C = crossbreed; DJ = Danish Jersey; RD = Red Danish; RW = Danish Holstein (Red and White).

nificantly different from 0, which indicated a high relation between the probability of testing ELISA positive and having a high SCC. The results indicate that among animals with high SCC, many will develop antibodies to MAP later if they have been exposed to or infected with MAP. The coefficient of determination indicates a good predictive ability of the model, where the independent variable explains more than 50% of the total variation of age at first positive ELISA. Linear regression techniques would have overestimated the R^2 (0.72 vs. 0.51). Fisher's exact test found a statistically significant difference between ELISA positivity and high SCC. The

relative risk of high SCC increased for ELISA-positive cows compared with ELISA-negative cows.

The cutoff value of 300,000 cells/mL was chosen arbitrarily. A sensitivity analysis, in which cutoff values of 200,000 and 100,000 cells/mL were used, suggested that the selected cutoff value did not influence the final outcome (data not shown). Robust regression estimates for each of the selected values were not significantly different, illustrating the robustness and reliability of the results.

The exploratory analysis of the 4 outcomes (ELISA positive/high SCC; ELISA positive/low SCC; ELISA negative/high SCC; ELISA negative/low SCC) indicates that MAP-positive animals have higher values of SCC. In previous cross-sectional studies (Nordlund et al., 1996; Hendrick et al., 2005), no significant association was found between MAP and SCC, but they did not include a longitudinal component, and the lack of association could be due to the 2 events not occurring simultaneously. Our study included repeated sampling over time from each cow, which improved the detection of MAP-infected animals and highlighted the time association between ELISA positivity and having high SCC.

Table 4. Parameter estimates for robust regression analysis of age at first positive ELISA/high SCC¹

Parameter	Estimate	SE	95% confidence limits ²	
			Lower	Upper
Intercept	0.339	0.019	0.302	0.377
Log age at first positive SCC	0.786	0.013	0.759	0.812

¹The model was based on 1,733 animals from 26 Danish dairy herds.

² $P < 0.0001$; model $R^2 = 0.51$.

Figure 3. Plots of predictions for the additive model with the independent variable age (in years) and the dependent variables (A) corrected optical density (ODc) and (B) SCC (in thousands). The 4 outcomes considered were as follows: ++ = ELISA positive/high SCC; +- = ELISA positive/low SCC; -+ = ELISA negative/high SCC; -- = ELISA negative/low SCC.

The Three Hypotheses

Three different scenarios were evaluated through the analysis of time of occurrence to MAP antibody positivity and having high SCC. A considerable proportion (40%) of cows first tested positive by ELISA. For example, based on the regression coefficients, an ELISA-positive animal at 4 yr of age may have had a high SCC 1.8 yr earlier; a 6-yr-old ELISA-positive animal may be expected to have experienced a high SCC 2.4 yr earlier. Although the events leading to mastitis are often of a multifactorial nature, the resistance of the host to mastitis pathogens may be influenced by the immune status of the animal. Therefore, a compromised immune response induced by progression of a MAP infection may predispose cows to mastitis, leading to increased SCC, as described by hypothesis 1. It is generally considered that antibodies are produced as the infection progresses from cell-mediated to humoral immune responses (Stabel, 2000), although antibodies occur during both immune phases. The duration of each process is not known, but is probably highly variable according to the results of Nielsen and Ersbøll (2006). A variable duration of the cell-mediated immune responses may explain the variation in whether an animal first tested positive in ELISA or in SCC, because a small amount of antibodies could be present during cell-mediated immune responses and elicit a positive ELISA. If that is the situation, hypothesis 2 or 3 is more likely. Only 12% of the ELISA-positive animals had low SCC. A large proportion (46%) of the animals had high SCC prior to a positive ELISA (hypothesis 2), suggesting that the process of mastitis infection may cause alterations in immune function that may lead to impaired cellular immunity to MAP, with progression of the infection and consequent antibody production. Because of the long incubation period of the infection, it can be hypothesized that a proportion of high-SCC animals never become ELISA positive because of withdrawal or incomplete observation at the end of the follow-up study. Depending on management practices and the condition of the immune system, cows can become infected with a mastitis pathogen and never become MAP positive if they have never been exposed to MAP.

A small proportion (14%) of the animals tested positive to MAP and high SCC simultaneously. Although the association between MAP and SCC was present, a third factor is probably affecting general immunity, resulting in both high SCC and antibodies to MAP (hypothesis 3).

The large variation in the distribution of first occurrence of MAP-positive assessment and high SCC indicates that none of the 3 hypotheses could be firmly excluded. Nevertheless, the variation in the results may

indicate that the effect of an unknown mechanism negatively affecting the general immune competences may lead to both SCC and MAP deterioration, thereby favoring hypothesis 3.

Implications for Use of Test Results

The study results indicate that there is an association between ELISA-positive measures and high SCC. Therefore, an increased risk that cows with high SCC become infectious with MAP should be considered by farmers and advisors. Test results can be used for targeting potentially infected and infectious animals. The management of MAP-infected animals should include procedures to avoid the use of milk from high-SCC cows.

CONCLUSIONS

The study results strongly suggest that the occurrence of MAP antibodies, and thereby cows with advanced MAP infections in the infectious stage, is associated with a high SCC. The high SCC occurred prior to an ELISA-positive assessment in most cows, but an ELISA-positive diagnosis preceded high SCC in 40% of the cows. Assuming that cows with antibodies to MAP are infectious via their milk, exposure of susceptible calves may result in MAP infection if milk-feeding strategies include the use of milk from high-SCC cows. It could not be determined whether the progression of a MAP infection leads to high SCC, whether a mammary infection leads to the evolution of MAP infections, or whether an outside factor was responsible for the progression of both, but the results suggest the latter to be likely.

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