Time to the occurrence of a decline in milk production in cows with various paratuberculosis antibody profiles

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ABSTRACT

Infection with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in dairy cattle often results in reduced milk production and premature culling. Some test-positive animals can live for years without being affected by infection, whereas others are test negative when they die from the infection. Our objective was to describe the deviation in milk production of cows with various MAP antibody profiles compared with their repeatedly test-negative herdmates in the same parity. Data were obtained from herds participating in the Danish control program on paratuberculosis, for which 4 annual MAP antibody ELISA of individual cows were performed per herd per year. A total of 136,489 ELISA results from 38,998 dairy cows in 64 herds were used along with 484,285 test-day records on energy-corrected milk (ECM) yield. Cows were divided into 6 antibody groups based on their repeated milk ELISA results: A0) repeated ELISA negative; A1) ELISA negative, but only once; A2) ELISA negative on the last 3 tests, but with 1 previous positive result; A3) ELISA negative on the last test, but with 1 or more previous positive results; A4) last sample was ELISA positive, but all previous were negative; and A5) at least the last 2 samples were ELISA positive. The expected test-day kilograms of ECM by herd and parity were estimated for cows in antibody group A0. Deviations from expected milk production were then assessed for cows in the other antibody groups relative to the time of the first test-positive ELISA result (D 0). Cows in groups A2, A3, and A5 produced approximately 0.5 kg of ECM/d more than cows in group A0 at 300 d before D 0. Cows in group A4 had a decline in milk production from d 300 before D 0, with daily milk production reduced by 5 kg of ECM at 200 d after D 0. Milk production of cows in group A5 was reduced by 2.5 kg of ECM at 300 d after D 0 compared with 300 d before D 0, whereas cows in groups A2 and A3 produced 0.5 kg of ECM

more than cows in group A0. The conclusions of the study were that 1) increasing the number ELISA tests increases the predictive value of ELISA for inference on milk production losses, 2) a combination of ELISA with assessment of observed milk production may be a valuable tool for decisions on culling, and 3) the declines in milk production attributable to MAP occurred over a long time period, and may not be realized by the herd manager without more advanced management tools such as the model proposed here.

Key words: antibody profile, milk enzyme-linked immunosorbent assay, milk production, paratuberculosis

INTRODUCTION

Paratuberculosis is a chronic infection caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). Control or certification programs are established in several countries (Kennedy and Nielsen, 2007), emphasizing the interest of the industry in controlling MAP infections. A primary reason is the economic losses that infection can cause in a herd. The major losses are incurred through reduced milk production and premature culling, with the latter resulting in increased cow replacement costs.

Some discrepancies exist in the literature concerning how much milk production is affected. Milk production was 20 and 17% less in the last lactation compared with the previous lactation among culled cows with clinical symptoms and among cows that were positive in a serum antibody ELISA with MAP infection confirmed by histology, respectively, in a Dutch study (Benedictus et al., 1987). Cows with a strongly positive serum ELISA result produced 1,364 kg less 305-d mature-equivalent milk (Lombard et al., 2005), and cows with positive milk ELISA produced 457 kg less milk per lactation (Hendrick et al., 2005) than ELISA-negative cows. Johnson et al. (2001) reported an increase ($P = 0.11$) in milk production among serum ELISA-positive cows compared with ELISA-negative cows. These studies were all cross-sectional, with diagnostic procedures carried out only at 1 time point per animal. Wilson et al. (1993) divided cows in a herd into MAP-infected and noninfected based on repeated fecal culture (FC). They

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found that FC-positive, first-parity cows in the beginning of lactation (≤ 100 DIM) produced 1.6% more milk compared with FC-negative cows, whereas FC-positive cows late (> 100 DIM) in the second, third, and fourth lactation produced 2.9, 8.2, and 8.4% less milk, respectively, compared with their FC-negative herdmates. These results suggest that animals that become test positive initially have a greater milk production, but later the test-positive animals produce less milk (equal to steeper lactation curves). Nevertheless, Wilson et al. (1993) did not assess the time from when an animal tested positive relative to when the decline in milk production occurred, and the study design was not appropriate for making inferences about when a production loss occurred and whether infected or test-positive animals had a greater milk yield early in the infection. To our knowledge, no studies have been performed to assess the period from test positivity to the occurrence of milk production losses, which may explain the discrepancies in results reported.

Because milk production losses associated with MAP infections can be one of the major motivators for controlling the infection, assessment of the time from a positive test to expected milk production losses would be needed for optimal timing of culling a test-positive animal. Results of the above-mentioned studies suggest that stage of infection affected the occurrence and magnitude of milk production losses. Nevertheless, losses need not be linked to the occurrence of antibodies in serum (Johnson et al., 2001; Hendrick et al., 2005) or to bacterial shedding (Wilson et al., 1993). Humoral immune reactions with the occurrence of antibodies generally characterize the progression of MAP infections, but some antibodies can be detected in the early stages of infection (Koets et al., 2001). Therefore, the antibody profile of a cow could be a better predictor of milk production losses than individual antibody measurements. The objective was to describe the time from detection of antibodies to MAP (as detected by a milk ELISA) to the occurrence of declines in milk production for cows with various antibody profiles.

MATERIALS AND METHODS

Data Collection

Data from the Danish control program on paratuberculosis (Nielsen et al., 2007) and the Danish milk recording scheme were extracted from the Danish Cattle Database and used for the analyses. Of the approximately 4,600 dairy herds in Denmark, 1,219 participated in the program on the date (May 8, 2008) of data extraction. Program herds fulfilling the following criteria were included: 1) more than 200 cows were

present in the herd on the date of data extraction, 2) herds were enrolled in the Danish milk recording scheme and had 11 annual milk production recordings, and 3) more than 90% of the cows in each herd were Danish Holsteins. These criteria were set to make the predicted milk yields of cows without MAP within a given herd as robust and precise as possible. Test-day records were excluded from cows with missing information on age at first calving, non-Holstein cows, when test days were > 305 DIM, and when test dates were > 3 yr old. The resulting data set consisted of 484,285 test-day records from 38,998 cows.

Herds in the MAP control program were tested 4 times yearly by using an in-house milk antibody ELISA (Nielsen, 2002). The milk samples tested were the same as those obtained in the milk recording scheme for assessment of milk yield, fat, protein, and SCC, and were a composite sample from the morning and evening milking. The test was based on a commercially available *Mycobacterium avium* ssp. *avium* purified protein derivative-antigen (Allied Monitor, Fayette, MO), and both IgG1 and IgG2 were detected by the test. Test responses ≥ 0.3 optical density corrected (OD_C) values were considered positive, but because of repeated testing, cows could be classified into 6 antibody groups based on their ELISA profile, rather than being evaluated based on a single test value only: **A0**) repeated ELISA negative based on a minimum of 2 samples; **A1**) ELISA negative, but only 1 sample was available; **A2**) ELISA negative on the last 3 samples, but with 1 previous positive result; **A3**) ELISA negative on the last test, but with 1 or more previous positive results, usually with interchangeable positive and negative reactions ("antibody fluctuator"); **A4**) the last sample was ELISA positive, but all previous were negative; and **A5**) at least the last 2 samples were ELISA positive. These 6 antibody groups were defined so that all cows were classified rule based. We used the same terminology and grouping as in Nielsen (2008), in which the time relationship between occurrences of MAP antibodies to shedding of MAP was studied. Cows with positive ELISA reactions were defined as belonging to the specific antibody groups on the date of the first positive result (**D 0**). Cows that shifted from one antibody group to another were classified based on the last result obtained, but the date of the first positive ELISA was retained as **D 0**. From the 38,998 cows, 136,489 test results were available: 7,694 cows with 1 test result, 7,027 cows with 2 test results, 7,526 cows with 3 test results, 6,712 cows with 4 test results, 4,621 cows with 5 test results, and 5,424 with 6 or more test results. The data included 23,098 cows in antibody group A0, 6,541 cows in antibody group A1, 728 cows in antibody group A2, 2,913 cows in antibody group

Table 1. Parity-specific distribution of herd-level averages of milk production and corrected optical density (OD_C) values¹

Item	Parity 1				Parity 2				Parity 3+			
	Mean	Q1	Q2	Q3	Mean	Q1	Q2	Q3	Mean	Q1	Q2	Q3
ECM, kg/d	27.8	24.4	27.9	31.3	32.5	27.5	32.5	37.4	33.4	27.8	33.4	39.1
ELISA test value, OD _C	0.05	0.00	0.00	0.00	0.12	0.00	0.00	0.10	0.15	0.00	0.10	0.20

¹Q1, Q2, and Q3 = quartiles 1, 2 and 3, respectively.

A3, 2,832 cows in antibody group A4, and 2,886 cows in antibody group A5.

Statistical Analyses

The statistical analyses were carried out in a 3-step process, in which the average kilograms of ECM yield were described for cows in parity 1, 2, and >2, and average OD_C values were described for cows in each of the antibody groups. The true prevalence was estimated based on the test prevalence, age of the individual, and age-specific estimates of sensitivity and specificity. The estimator described by Sergeant and et al. (2008; available at <http://parafree.vetinst.dk/parafree/content.php?page=parafree>) was used for the estimations. The predicted kilograms of ECM were then estimated for each cow in a given parity in a given herd for all cows that were repeatedly negative in ELISA (cows in antibody group A0). Finally, this expected milk yield was used to estimate deviations in the milk yield of cows of other antibody groups. These analyses were performed as described below.

Descriptive Statistics. Test-day milk yield was recorded 11 times yearly in routine milk recordings, at which time kilograms of milk, percentage of fat, and percentage of protein were determined. The test-day ECM (ECMT) yield was calculated by using the formula

$$\begin{aligned} \text{kg of ECMT} = & [\text{kg of milk} \times (383 \times \text{fat}\% + 242 \\ & \times \text{protein}\% + 780.8)] / 3,140. \end{aligned} \quad [1]$$

For each of the 3 parity groups, 1, 2, and >2, the mean, median, first quartile, and third quartile kilograms of ECMT and OD_C were calculated. Subsequently, kilograms of ECMT were estimated as a function of DIM for each parity group by using a generalized additive model (Hastie and Tibshirani, 1990) using a B-spline smoother with 10 degrees of freedom with PROC GAM in SAS, version 9.1.3 (SAS Inst. Inc., Cary, NC). The resulting average lactation curves were used to determine the shape of the lactation curves for the estimations of expected milk yield.

Average antibody profiles for cows in each antibody group were described by using the same approach as for ECMT, with OD_C as a function of time relative to D 0. For cows in antibody group A0, D 0 was the median between the first and last test dates. The median test date was used because that date would be in the middle of the study period, and a uniform fixed point was needed. For cows in antibody group A1, only 1 observation was available per cow, and these cows were excluded because all observations would be at D 0.

Prediction of Kilograms of ECMT in Antibody Group A0. The lactation curves suggested a peak milk yield between 40 and 65 DIM, and lactation curves were fitted similar to those described by Bennedsgaard et al. (2003), where peak milk yield was 60 DIM. The predicted milk yield for cows in antibody group A0 was estimated by using the 3-level model for separate parity strata, 1, 2, and >2:

$$\begin{aligned} \text{ECM}_{ijk} = & \beta_0 + \beta_1 \text{DIMun60}_{ijk} + \beta_2 \text{DIM60}_{ijk} \\ & + \beta_3 \text{AC1}_{jk} + \beta_4 \text{Twin}_{jk} + \beta_5 \text{Calving}_{jk} \\ & + \beta_6 \text{Season}(\text{Year})_{jk} + \varepsilon_{ijk}, \end{aligned} \quad [2]$$

where $\beta_0 = \beta_{000} + \nu_{00k} + \mu_{0jk}$, $\beta_1 = \beta_{1000} + \nu_{100k} + \mu_{10jk}$, $\beta_2 = \beta_{2000} + \nu_{20k0} + \mu_{20jk}$, and $\nu_{00k} \sim N(0, \tau_{00k})$, $\mu_{0jk} \sim N(0, \tau_{0jk})$, $\nu_{100k} \sim N(0, \tau_{100k})$, $\mu_{10jk} \sim N(0, \tau_{10jk})$, $\nu_{20k0} \sim N(0, \tau_{20k0})$, $\mu_{20jk} \sim N(0, \tau_{20jk})$, and $\varepsilon_{ijk} \sim N(0, \sigma_{ijk})$.

The variable ECM_{ijk} was the kilograms of ECM on the *i*th test day of the *j*th cow in the *k*th herd; DIMun60_{ijk} was the *i*th DIM of the *j*th cow in the *k*th herd for DIM 1 to 60. For DIM >60, DIM60 takes the value 0; DIM60_{ijk} was the *i*th (DIM – 60)/245 of the *j*th cow in the *k*th herd for 60 to 305 DIM. For values <60 DIM, DIM60 takes the value 0; AC1_{jk} was the age at first calving for the *j*th cow in the *k*th herd (this effect was included only for first-parity cows); Twin_{jk} was the effect of the *j*th cow giving birth to a twin in the *k*th herd; and Calving_{jk} was the effect of calving problems of the *j*th cow in the *k*th herd based on recordings of the calving made by the farmer. Calving was classified as yes or no, where yes was difficult calvings and veterinary-assisted calvings; and Season(Year)_{jk} was a nested effect of the season (1: January to March;

Figure 1. Parity-specific kilograms of ECM as a function of DIM for all cows in the 64 Danish dairy herds.

2: April to June; 3: July to September; 4: October to December) in a study year (2005, 2006, 2007, or 2008) on the j th cow in the k th herd.

The variable β_0 can be separated into an overall mean (β_{000}) that represents the average across-herd milk yield at 60 DIM and a contribution from the individual herds (ν_{00k}), and a contribution from the j th cow within the k th herd (μ_{0jk}). The variable ν_{0k} was the mean ECM in the k th herd; μ_{0jk} was the mean ECM of the j th cow in the k th herd; β_0 was the overall mean of ECM at the intercept; β_1 , β_2 , and β_3 were the fixed linear regression coefficients of DIM60, DIMun60, and AC1, respectively; β_4 , β_5 , and β_6 were class effects of the Twin, Season(Year), and Calving, respectively; ν_{2k} was the random linear regression coefficient of DIM60 $_{ijk}$; μ_{2jk} was the random linear regression coefficient of DIM60 $_{ijk}$; and ε_{ijk} was the a random residual component assumed independent, identically distributed normal, $N(0, \sigma^2)$.

Deviation from Predicted Kilograms of ECM for Cows from Various Antibody Groups. The predicted kilograms of ECM of cows in antibody group A0 from the same parity in the k th herd was used as the expected kilograms of ECM of a given cow in the same parity and in the same herd. Also included in the predictions were the remaining covariates described in equation [2]. The deviation in kilograms of ECM from the expected milk yield was calculated for cows in antibody groups A2 to A5. The D 0 was used to calculate the time from the ECM test date to a positive ELISA test: $T_{Diff} = \text{milk recording test date} - D 0$.

Deviations of the kilograms of ECM were then estimated as a function of T_{Diff} . The model used for each of these groups was

$$\begin{aligned} \text{Deviation kg of ECM} = & \beta_0 + \beta_1 T_{Diff} + \beta_2 T_{Diff}^2 \\ & + \beta_3 T_{Diff}^3 + \beta_4 T_{Diff}^4 + \beta_5 T_{Diff}^5 + \varepsilon. \end{aligned} \quad [3]$$

The results were visualized by plotting the deviation in kilograms of ECM relative to T_{Diff} for each antibody group.

RESULTS

The parity-specific distributions of test-day kilograms of ECM and OD_C-values from ELISA are given in Table 1. Averages of parity-specific kilograms of ECM as a function of DIM are shown in Figure 1. Average ELISA profiles for antibody groups A0 and A2 to A5 are shown in Figure 2. The estimated distribution of within-herd true prevalences was as follows: minimum: 0%; first quartile: 1%; median: 9%; third quartile: 20%; and maximum: 52%.

The expected kilograms of ECM for cows in antibody group A0 were calculated and are represented by the value 0 kg of ECM in Figure 3. Deviations from 0 kg of ECM for cows with other antibody profiles are also shown in Figure 3. Mean milk yield of cows in antibody groups A2 and A3 were >0.5 kg of ECM greater per test date from 400 d before D 0 until 300 d after D 0. In

Figure 2. Average optical density (OD) value in ELISA for cows in the antibody groups relative to the date of the first positive ELISA test (antibody groups A2 to A5) or relative to the difference between the first and last negative ELISA test result (antibody group A0).

addition, group A5 had a greater milk yield until 100 d before D 0. From -100 to $+300$ d relative to D 0, test-day milk yield decreased 2.3 kg of ECM in antibody group A5. Test-day milk yield decreased 4 kg of ECM in antibody group A4, beginning approximately 300 d before D 0 and with an apparent maximum decrease around 100 d after D 0.

Approximately 100 d after D 0, the uncertainty (visualized through the 95% confidence band) related to the decline in milk production for group A4 was much greater than previously, indicating that the number of observations from this period was reduced, probably because of culling or death of the animals. The uncertainty associated with the deviation in milk production for cows in antibody group A5 was smaller compared with the uncertainty of cows in antibody group A4 at 200 d after D 0.

DISCUSSION

The results showed that milk production was significantly reduced for cows when the last ELISA result was positive. The onset of this production loss occurred 300 d before the date the antibodies occurred, and the deviation from expected milk yield continued to increase after the cow had become antibody positive. Interestingly, cows that had repeated positive ELISA results had a less pronounced deviation in milk production loss compared with those with only 1 unconfirmed result. Two likely explanations are discussed further. It is likely that some cows cope better with the infection than others for a while, maybe because of a feed ration that supports the immune system of the cow (Stabel and Goff, 2004). Such a cow may be able to endure the infection for a longer time, but will eventually experience a decline in milk production. Another possible ex-

Figure 3. Deviation from predicted milk yield as a function of time from first positive ELISA test for cows from antibody groups A2 to A5, relative to the expected milk yield based on cows from antibody group A0 in the k th herd in parity groups 1, 2, or > 2 . Shaded areas represent 95% confidence intervals for each data point.

planation is that the proportion of false-positive ELISA reactions was greater among cows that were repeatedly ELISA positive, thus diluting the effect caused by MAP infections. The latter explanation seems less likely, because the production loss occurred eventually, and the uncertainty associated with this loss was moderate.

The finding that all cows that become positive have a greater milk production 200 to 400 d before becoming test positive supports the findings of Wilson et al. (1993) and suggests that greater producing dairy cows were more likely to be test positive and eventually experience reduced milk production. These results would explain the discrepancies in the significance and magnitude of milk production losses described in previous studies (Johnson et al., 2001; Hendrick et al., 2005; Lombard et al., 2005). Our results provide support for the validity of our statistical approach, which allowed such patterns, in contrast to a model that attempts to estimate the contrasts between categories directly. We chose to use this model because the data would define the onset of the "disease process" (i.e., losses in milk production).

Culling based on 1 test-positive result is generally discouraged in the Danish control program, unless other information (such as a decline in milk production) is used in combination with the ELISA results (Nielsen et al., 2007). The results from the current study suggest that cows in antibody groups A4 and A5 could be potential candidates for culling based on their milk production, whereas cows in groups A2 and A3 were not, although, in a control plan on paratuberculosis, the risk of shedding of MAP from these cows should be considered to reduce the transmission of MAP. Cows in antibody group A2 previously had a low probability of shedding MAP on a given day in the following year (2 to 3%), whereas the probability increased for cows in groups A3, A4, and A5 (Nielsen, 2008). Cows in groups A4 and A5 had high risks of shedding MAP, either on the test date or in the following test period. They may thus be contributing to the transmission of MAP, and culling decisions should include this aspect. Cows in group A3 may not be obvious candidates for culling from a milk loss point of view. Therefore, an alternative to culling could be to ascertain that the cow does not transmit MAP to susceptible cattle in the herd. A potential negative effect on the production economy caused by culling based on false-positive test reactions could be reduced.

A weakness of the current study was the use of a nonperfect test, which was neither 100% sensitive nor 100% specific. This is a general problem in studies on MAP. Confirmatory testing (e.g., using FC) is not used in the Danish program, although the possibility exists. Confirmatory testing could have reduced the number

of false positives, but the number of false negatives would have increased. From a practical point of view, it was considered important to mimic the situation of the farmers. Because the production loss occurred before test positivity, and because a FC result can be achieved only 2 to 4 mo after the ELISA result, the usefulness of FC would be limited. In addition, an animal might be in a meager body condition if her milk production was already affected. Therefore, in practice a combination of deviation in milk production compared with the expected milk yield, combined with a test-positive result would be a much better (timely) basis for decisions on culling than an ELISA result combined with a confirmatory FC result.

A major strengths of this study were the availability of data from a huge population, which generally resulted in low uncertainty of the deviations in milk production, and the use of the predictive value of the individual cow in the individual herd. The variation that did exist was used to further explain the results. The results can be used directly by farmers to predict the fate of a cow, although better models may be developed when more test-day records become available. Nevertheless, the best (and least expensive) option to reduce the probability of uncertainty is the availability of more frequent ELISA tests per cow. The results can be used in the development of more precise models for estimating milk production losses on the herd and national level (including sires), because divisions into antibody groups are strong predictors for milk production losses. Another important finding was the long time span from the initial production loss to the maximum production loss. Such losses may not be realized by the herd manager without more advanced management tools, such as the model proposed here. Illustration of this chronicity problem has not been possible based on a sample representative of a major dairy population. It is important to note that cows with various antibody profiles experience different production losses, which is probably due to the capability of some cows to control the infection even when antibodies have been produced (Koets et al., 2001).

CONCLUSIONS

Major milk production losses for cows with certain well-defined paratuberculosis antibody profiles can commence 300 d before the first positive antibody test. Cows with fluctuating antibody profiles do not experience losses. Predictions of milk production loss from paratuberculosis are substantially more precise and useful when they are based on repeated test results compared with single test results.

REFERENCES

- Benedictus, G., A. A. Dijkhuizen, and J. Stelwagen. 1987. Economic losses due to paratuberculosis in dairy cattle. *Vet. Rec.* 121:142–146.
- Bennedsgaard, T. W., C. Enevoldsen, S. M. Thamsborg, and M. Vaarst. 2003. Effect of mastitis treatment and somatic cell counts on milk yield in Danish organic dairy cows. *J. Dairy Sci.* 86:3174–3183.
- Hastie, T. J., and R. J. Tibshirani. 1990. *Generalized Additive Models*. Chapman and Hall, London, UK.
- Hendrick, S. H., D. F. Kelton, K. E. Leslie, K. D. Lissimore, M. Archambault, and T. F. Duffield. 2005. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. *J. Am. Vet. Med. Assoc.* 227:1302–1308.
- Johnson, Y. J., J. B. Kaneene, J. C. Gardiner, J. W. Lloyd, D. J. Sprecher, and P. H. Coe. 2001. The effect of subclinical *Mycobacterium paratuberculosis* infection on milk production in Michigan dairy cows. *J. Dairy Sci.* 84:2188–2194.
- Kennedy, D. J., and S. S. Nielsen. 2007. Report from the first IDF ParaTB Forum. *Bull. Int. Dairy Fed.* 410:3–7.
- Koets, A. P., V. P. M. G. Rutten, M. de Boer, D. Bakker, P. Valentin-Weigand, and W. van Eden. 2001. Differential changes in heat shock protein-, lipoarabinomannan-, and purified protein derivative-specific bovine *Mycobacterium avium* subsp. *paratuberculosis* infection. *Infect. Immun.* 69:1492–1498.
- Lombard, J. E., F. B. Garry, B. J. McCluskey, and B. A. Wagner. 2005. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. *J. Am. Vet. Med. Assoc.* 227:1975–1981.
- Nielsen, S. S. 2002. Variance components of an enzyme-linked immunosorbent assay for detection of IgG antibodies in milk samples to *Mycobacterium avium* subsp. *paratuberculosis* in dairy cattle. *J. Vet. Med. B* 49:384–387.
- Nielsen, S. S. 2008. Transitions in diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* infections in cattle. *Vet. Microbiol.* 132:274–282.
- Nielsen, S. S., Ø. R. Jepsen, and K. Aagaard. 2007. Control programme for paratuberculosis in Denmark. *Bull. Int. Dairy Fed.-Fed. Int. Laiterie* 410:23–29.
- Sergeant, E. S., S. S. Nielsen, and N. Toft. 2008. Evaluation of test-strategies for estimating probability of low prevalence of paratuberculosis in Danish dairy herds. *Prev. Vet. Med.* 85:92–106.
- Stabel, J. R., and J. P. Goff. 2004. Efficacy of immunologic assays for the detection of Johne's disease in dairy cows fed additional energy during the periparturient period. *J. Vet. Diagn. Invest.* 16:412–420.
- Wilson, D. J., C. Rossiter, H. R. Han, and P. M. Sears. 1993. Association of *Mycobacterium paratuberculosis* infection with reduced mastitis, but decreased milk production and increased cull rate in clinically normal dairy cows. *Am. J. Vet. Res.* 54:1851–1857.