J. Dairy Sci. 108:11303–11316
https://doi.org/10.3168/jds.2025-26761

Patterns of *Mycoplasma bovis* antibodies in cows and calves in Swedish dairy herds, and testing strategies to detect seropositive herds

E. Hurri,[1,2]* C. W. R. Compton,[3] K. Alvåsen,[1] and M. Tråvén[1]
[1]Department of Clinical Sciences, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
[2]Department of Animal Health and Antimicrobial Strategies, Swedish Veterinary Agency (SVA), SE-751 89 Uppsala, Sweden
[3]School of Veterinary Science, Massey University, Palmerston North 4442, New Zealand

ABSTRACT

Mycoplasma bovis causes severe diseases among cattle. Sweden has a favorable situation for control of this disease, with a low prevalence of *M. bovis* infected and seropositive herds detected only in the southern parts of the country. To prevent the spread of the infection, analyzing antibody levels is a cost-effective method to determine herd pathogen exposure status. In this study, our aims were to monitor the antibody dynamics in infected herds over time using IDvet ELISA, to both evaluate risk-based sampling and investigate the effect of *M. bovis* exposure on health and production. We visited and sampled 35 dairy herds, 31 of which were sampled at least 2 times and 26 sampled 4 times and followed for 2 yr. The patterns of herd seroprevalence varied depending on the status before the herd's entry into the study and remained relatively stable at the herd level, although antibody status could differ among age groups. Overall, herds with high exposure prevalence (75%–100% positive cows, n = 13/26), and herds with low exposure prevalence (<25% positive cows, n = 5/26), maintained their exposure status throughout the study. The changes in status observed within the herds, both among calves and cows, included transitions from positive to negative, as well as from negative to positive. In 5 herds, the calf group transitioned from positive to negative, while in 1 herd, the reverse occurred. Three herds exhibited an increase in antibody levels; in 2 of these herds, the cows transitioned from negative to positive, and in 1 herd with positive cows, the calves shifted from negative to positive. A cost-effective test strategy to find likely infected herds involved sampling bulk tank milk and cows with a high SCC, which gave a 90% probability of locating infected herds by the second sampling. Milk production was reduced by 404 kg (1.3 L/d, $P = 0.012$) in cows positive for *M. bovis* antibodies. Therefore, controlling the spread of *M. bovis* infection will likely have a positive effect on reducing income loss for dairy herds in Sweden.
Key words: cattle, longitudinal, serology, *Mycoplasmopsis bovis*

INTRODUCTION

Mycoplasma bovis is an important bovine pathogen worldwide. The bacterium is associated with various diseases in cattle, including mastitis, arthritis, pneumonia, and otitis media (Nicholas and Ayling, 2003). Infections with *M. bovis* are difficult to treat and often become chronic or result in the animal being culled, leading to both economic losses for the dairy industry and implications for animal welfare (Maunsell et al., 2011).

In 2011, cases with *M. bovis*, involving both calves with pneumonia and cows with mastitis, were diagnosed in southern Sweden (Ericsson Unnerstad et al., 2012). Moreover, in 2019, a national screening of antibodies to *M. bovis* using bulk tank milk (**BTM**) samples from all Swedish dairy herds reported a prevalence of 4.8% (Hurri et al., 2022). The screening revealed large regional differences, with all seropositive herds being situated in southern Sweden, indicating a rather slow spread of *M. bovis* from the first detection in the southernmost part of the country (Ericsson Unnerstad et al., 2012). The low prevalence is an incentive to control this disease and there is currently a need for diagnostic tools and strategies to achieve this. Detection of infected cattle, including subclinically infected animals, is essential to prevent both introductions into naïve herds and outbreaks (Caswell and Archambault, 2007; Maunsell et al., 2011). Antibody testing of individual milk or serum samples has the potential to be a useful tool for detecting *M. bovis* carriers, when considering that intermittent shedding limits the detection of the bacterium by other pathogen-based tests (Biddle et al., 2003; Hazelton et al., 2018a, 2020a).

A combination of test results from serology, PCR, and culture, along with an evaluation of clinical signs and

Received April 14, 2025.
Accepted June 17, 2025.
*Corresponding author: emma.hurri@slu.se

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

herd history, is necessary to accurately characterize the infection status of a herd (Parker et al., 2018). For monitoring herds in a control program, a Finnish study suggested regular testing of antibodies with a longitudinal collection of sera and BTM, and nasal swabs for PCR from youngstock. The monitoring should also include *M. bovis* PCR analysis on samples from all cows with mastitis and from calves with pneumonia (Vähänikkilä et al., 2019). In the Finnish control program for *M. bovis*, herds are visited regularly for surveillance of clinical signs, and samples are collected 2 times a year from BTM for antibody testing, and nasal swabs from calves for PCR (Haapala et al., 2021). In the eradication program against *M. bovis* in New Zealand, antibody testing of BTM is being carried out for surveillance with samples taken from dairy herds monthly or every 2 wk (Ministry of Primary Industries, 2023). A BTM antibody-positive test result will lead to on-farm testing of all the cows or a percentage of the cows for herds consisting of more than 220 cows (personal message January 2024, Olivia Kingston, BTM program manager, OSPRI, Wellington, New Zealand). However, testing the whole herd to detect cows with *M. bovis* infection, following resolution of a mastitis outbreak, is highly expensive with low diagnostic yield (Hazelton et al., 2020b). A risk-based sampling to locate infected cows could, however, be more effective, using SCC and udder health history to select cows for sampling in a risk-based approach. Ultimately, the testing strategy employed will depend on the resources available and its intended objective. For example, a testing strategy for identifying likely infected herds in low prevalence or endemic populations will be quite different to that in a region or country whose objective is elimination.

In recent studies investigating herds following an outbreak with *M. bovis* mastitis, antibodies remained in the herds after the clinical symptoms had declined (Vähänikkilä et al., 2019; Penterman et al., 2022). Petersen et al. (2018) measured antibodies in cows who were part of outbreak herds in Denmark using the ELISA Bio K 302 (Bio-X Diagnostics, Belgium) and could only detect antibodies in milk from cows with mastitis. However, in a study comparing the Bio-X ELISA with the more sensitive IDvet ELISA (Innovative Diagnostics), the latter detected antibodies in milk samples from clinically normal cows in *M. bovis* infected herds (Petersen et al., 2020). In Finland, Vähänikkilä et al. (2019) followed infected herds for 2 yr and reported that 6 out of 19 farms became low risk as the infection was resolved after advice of culling the infected cows and keeping the calves separate from the cows. Another longitudinal study followed dairy heifers that were exposed to *M. bovis* preweaning, 72% (n = 289/400) of which had antibodies at weaning, and 18% (n = 65/356) had antibodies remaining at sampling 1 mo postcalving (Hazelton et al., 2020a). In addition, 1 of 50 heifers also shed the bacterium postcalving. *Mycoplasma bovis* infection is challenging to eradicate once it has become established within the herd, and at present, there is no test to ascertain that a herd or animal is free of *M. bovis*. A herd with antibodies is at risk of having animals that are asymptomatic carriers capable of shedding the bacterium (Vähänikkilä et al., 2019; Hazelton et al., 2020a; Penterman et al., 2022). A high level of biosecurity to prevent the introduction of *M. bovis* into the herd is therefore imperative (Haapala et al., 2021).

The relatively new commercial ID screen indirect ELISA test (IDvet, Grabels, France) has high sensitivity (92.5%–93.5%) and specificity (98.6%–99.3%) for detection of antibodies in serum. Evaluation has been performed on IDvet ELISA and Bio-X ELISA with western blot as the gold standard (Andersson et al., 2019) and by using Bayesian latent class analysis (Veldhuis et al., 2023). The IDvet ELISA is a suitable tool for herd-level control and surveillance purposes, as a small sample of cows provides a good indication of the exposure status of the age group as a whole (Petersen et al., 2020; Marquetoux et al., 2023). Milk samples can replace serum samples in adult cows, as demonstrated by a similar test performance with the IDvet ELISA on serum and milk (Petersen et al., 2020). Antibodies can be detected 2 to 3 wk after experimental infection in calves (Zhang et al., 2014). However, studies investigating the duration that antibodies remain above a cutoff point to define a positive status are limited. At herd level, antibodies measured with an in-house MilA ELISA developed by Wawegama et al. (2014) persisted in the serum of certain cows for at least 1.5 yr following an outbreak within the herd (Vähänikkilä et al., 2019). Further, an evaluation of best practice for a diagnostic test strategy with the IDvet ELISA in dairy herds is currently lacking.

Somatic cell count is an indirect measure of intramammary infection status in cows. Cows with *M. bovis* clinical mastitis had an elevated SCC of 650,000 cells/mL during the month that mastitis was detected (Wilson et al., 1997). Subclinical *M. bovis* mastitis is associated with lower milk yield, and higher SCC and *M. bovis* seropositive cows produced less milk than seronegative cows (Uhaa et al., 1990; Timonen et al., 2017). The symptoms of clinical *M. bovis* mastitis are nonspecific although often more than one-quarter are affected, there is a drastic decrease in milk production while signs of systemic illness are relatively mild, and the symptoms are unresponsive to standard treatments (Maunsell et al., 2011). Due to the poor prognosis for intramammary infection with *M. bovis* and the risk of transmission, the recommendation is generally to cull these cows (Nicholas et al., 2016).

In Sweden, *M. bovis* was diagnosed relatively recently in dairy herds compared with other European countries.

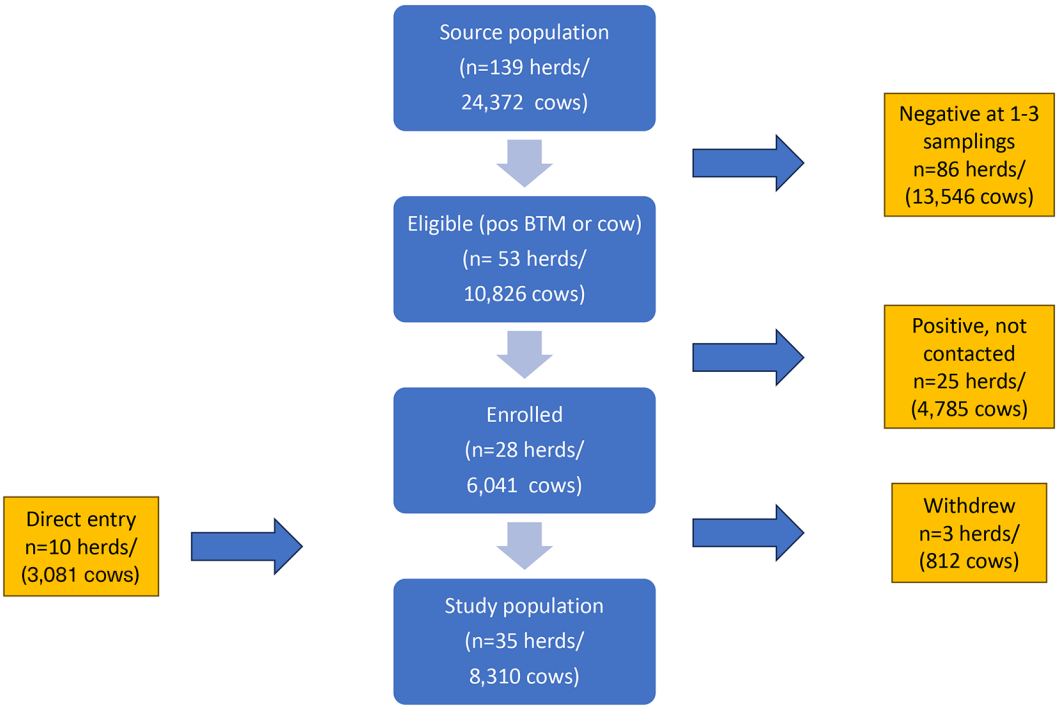

Figure 1. Selection of the study population. Eligible herds had either a *Mycoplasma bovis* antibody-positive (pos) bulk tank milk (BTM) or an antibody-positive primiparous cow. The herds with direct entry had a PCR-positive *M. bovis* diagnosis either in BTM or in calves. Two of these herds had unknown *M. bovis* status and had bought animals from *M. bovis*–positive herds.

A collaboration between industry and animal health organizations began in 2019, working together to prevent the spread of *M. bovis* among cattle herds. In this work, it is essential to locate infected herds and infected animals in a cost-effective manner. The aim of this study was to evaluate a risk-based sampling strategy in infected herds. The specific objectives were to (1) describe patterns of *M. bovis* milk and serum antibody test results over a 2-yr period, (2) calculate the sensitivity of different test strategies to detect *M. bovis* antibody-positive herds, and (3) estimate the effect of individual *M. bovis* antibody test results on the health and production of dairy cows.

MATERIALS AND METHODS

Between June 2019 and March 2023, a longitudinal study was performed, which involved a cohort of 35 Swedish dairy herds. The participating herds tested positive for *M. bovis* before entering this study.

Study Population

The source population was herds participating in another longitudinal study (Hurri et al., 2025). The eligible herds had a seropositive sample from BTM or milk, or both, from primiparous cows (**PP**). Location was considered for recruitment to be able to include herds from all represented regions, but it was not a strict inclusion criterion. Of the 53 positive herds, 28 herds were enrolled in the present study, although 3 herds withdrew before the herd visits started (Figure 1). The remaining 25 herds had at start either an antibody-positive BTM (56%, n = 14) or at least 1 antibody-positive PP cow (44%, n = 11). For the 10 additional herds that entered the study, 8 had a positive *M. bovis* diagnosis by PCR and 2 herds had introduced cattle from a positive herd and were determined antibody-positive in this study. The positive PCR diagnosis was from either a BTM sample (n = 2) in the biosecurity program (Säker Livdjurshandel, Växa), or a positive PCR diagnosis in calves (n = 6), tested in a research project or in connection with animal sales. This resulted in a total of 35 herds being visited, sampled, and providing animal health and production data. In 10 herds, the sampling was completed by the first author, and, for the remaining herds, the sampling was completed by local herd veterinarians. The time from the *M. bovis* diagnosis before enrollment in the study (antibodies or PCR) to the first herd visit with sampling, in the present study, was a median of 125 d (interquartile range [**IQR**] 102–183, minimum 16, maximum 686; Supplemental Table S1, see Notes).

Herd characteristics, including herd size and milk production, were aggregated in a 12-mo period before enrollment in the study, that is, September 1, 2018, to

Table 1. Herd characteristics for the 35 herds participating in this study, showing median, interquartile range (IQR), minimum, and maximum[1]

Variable	n	Median	IQR	Minimum	Maximum
Milk production[2]	29	10,846	10,301–11,629	8,973	12,625
Herd size (no. of cows)	35	150	97–327	32	741
BTM SCC[3]	34	247	208–296	82	727
Early calf mortality[4,5] (1–60 d)	29	4	2–7	0	19
Late calf mortality[4,5] (2–6 mo)	29	1	0–3	0	7
Youngstock mortality[4,5] (6–15 mo)	29	1	0–2	0	7
Cow mortality[4,5]	29	7	5–9	1	14
Culling of first parity cows in early lactation[5] (0–90 d)	29	4	2–7	0	15
Culling due to udder diseases[5]	29	8	7–12	1	22
Culling due to hoof and leg diseases[5]	29	4	3–5	0	9
Culling due to any reason including cow mortality[5]	29	40	34–44	25	54
Veterinary-treated clinical mastitis[5]	29	8	4–10	0	22
Veterinary-treated hoof and leg diseases[5]	29	2	1–4	0	13
Calving interval[6] (mo)	29	13	13–13	12	15
Age at first calving (d)	29	812	772–837	728	971
Breed[7]					
Swedish Red	4 (11)				
Swedish Holstein	15 (43)				
Mixed Red/Holstein	9 (26)				
Other breeds	1 (3)				
Unknown	6 (17)				
Region[7]					
Halland	8 (23)				
Kalmar	2 (6)				
Kronoberg	3 (9)				
Skåne	8 (23)				
Västra Götaland	8 (23)				
Östergötland	6 (17)				

[1]Data were withdrawn from the DHI database for 12 mo aggregated from September 1, 2018, to August 31, 2019 (Växa Sverige, 2019).

[2]Mean production per cow per 12 mo in kilograms of ECM.

[3]Bulk tank milk SCC in 1,000 cells/mL, arithmetic mean of 12 monthly measurements.

[4]Mortality includes death and euthanasia.

[5]Cases per 100 animals at risk per year.

[6]Mean interval between latest calving and the calving before that, for all cows from second lactation giving birth during the 12-mo period.

[7]Values presented as n (%).

August 31, 2019 (Table 1). Of the 35 herds included in the study, 83% (n = 29) were affiliated with the DHI program. All 35, however, had data regarding herd size.

Sampling

The sampling scheme was planned as 4 visits to each farm with ~6 mo intervals. At each visit, the veterinarian and the farmer or staff at the farm collected a sample of BTM, milk from 10 PP cows and milk from 10 multiparous cows (**MP**), blood samples from 20 calves/youngstock (2–10 mo old), and nasal swabs from 10 of these calves/youngstock. In 14 herds the BTM sample was missing, but BTM results from the automatic sampling collected by the milk quality laboratory in another study were used instead (Hurri et al., 2025). Veterinarians and farmers were asked to collect the number of samples (n = 20) from cows with a high SCC or a recent mastitis case. At the following herd visits, the veterinarian and farmer were asked to resample up to 10 cows that were antibody-positive at the previous sampling. Nasal swabs were collected from calves that had untreated symptoms of respiratory disease or otitis or arthritis if present, otherwise, the nasal swabs were taken from healthy calves. Milk samples were collected in 10-mL sterile test tubes. Blood samples were collected by venipuncture in 6-mL plain Vacutainer tubes (Next2Vet, Sweden). The nasal swabs were collected with ESwab (Copan, Murrieta, CA). The samples were then sent by postal service at ambient temperature to the Swedish University of Agricultural Sciences (**SLU**), Uppsala. Milk and centrifuged serum samples were stored at −20°C until analysis. The nasal swabs for PCR analysis were sent by postal service at ambient temperature to the Swedish Veterinary Agency, Uppsala, and analyzed the same day they arrived at the laboratory.

Table 2. Estimation of costs for sampling and probability of detecting a positive herd by *Mycoplasma bovis* antibody analysis in blood from calves/youngstock, milk from individual cows, or bulk tank milk (BTM)

	Calves/youngstock (n = 20)		Cows (n = 20)		BTM	
Sampling occasion	Estimation of costs[1] (SEK [€; USD])	Probability[2] (%)	Estimation of costs[1] (SEK [€; USD])	Probability (%)	Estimation of costs[1] (SEK [€; USD])	Probability (%)
1	3,200 (291; 329)	50	2,200 (200, 226)	77	185 (17, 19)	59
2	6,400 (582; 659)	61	4,400 (400, 453)	90	370 (34, 38)	67
3	9,600 (873; 988)	65	6,600 (600, 679)	96	555 (50, 57)	77
4	12,800 (1,163; 1,317)	65	8,800 (800, 906)	100	740 (67, 76)	79

[1]Estimation of costs for sampling presented as SEK (€; USD).

[2]Probability of detecting 1 or more positive samples.

Laboratory Analysis

Milk and serum samples were analyzed for antibodies of *M. bovis* with ID screen indirect ELISA (IDvet, Grabels, France) at the Department of Clinical Sciences, SLU, according to the manufacturer's instructions. The relative amount of antibodies present in the samples was calculated as (sample optical density [**OD**] − negative control OD)/(positive control OD − negative control OD) × 100 (sample/positive [**S/P**]%). The milk samples were analyzed with the overnight incubation protocol, and the cutoff for a positive sample was set to S/P% ≥30%, as recommended by the manufacturer. The cutoff for a positive serum sample was set to S/P% ≥60%, as recommended by the manufacturer. The DNA from nasal swabs was extracted using IndiMag Pathogen kit (Indical Bioscience GmbH, Leipzig, Germany). The PCR analysis for *M. bovis* was performed using the primers and probe described by Sachse et al. (2010). The reactions consisted of PerfeCTa qPCR Toughmix (Quantabio, Beverly, MA), 500 n*M* each primer, 100 n*M* probe, and 2 µL of DNA template in a total volume of 15 µL. The amplification was performed using ABI 7500 Fast real-time PCR platform (Applied Biosystems, Waltham, MA) and the following temperature profile: 50°C for 10 min, 95°C for 3 min, 45 cycles of 95°C for 3 s, and 60°C for 30 s. Samples with a threshold cycle value of 40 or below were considered positive for *M. bovis*.

Test Strategy Evaluation and Costs

The probability of finding positive herds by testing different age groups was calculated by evaluating 7 different test strategies at 1, 2, 3, or 4 occasions. The test strategies for antibodies were (1) calves only, (2) cows only, (3) cows and calves, (4) BTM only, (5) BTM and calves, (6) BTM and cows, and (7) BTM, cows, and calves. Each herd was either negative (0 = no positive samples) or positive (1 = 1 or more positive samples) at each level of the 7 test strategies at herd visit 1. For the following herd visits, the test result from the prior visit or visits was added to the test result. The number of positive herds was then divided by the number of tested herds, and this was defined as the sensitivity of the test strategy to detect test-positive herds. Furthermore, the test strategy assumed that all study herds were exposed at the time of their enrollment based on prior testing history, that all study herds remained infected and potentially capable of having one or more antibody-positive animals throughout the study period, and that the antibody test had 100% specificity (zero false-positive results). The test strategy also assumed that because different age groups might be exposed differently, the risk-based sampling strategies might vary in their sensitivity to determine the herd exposure status (≥1 antibody test-positive in any age group defined as herd positive). This approach was taken because of the limitations with all available diagnostic tests, presence of subclinical and carrier animal states, and limited study resources. In the calculation of costs, we have included the laboratory costs for the ELISA analysis and the costs for the collection of samples. Collecting a BTM sample is the cheapest alternative, and collecting blood from calves/youngstock is the most expensive (Table 2). The cost for laboratory ELISA analysis is set at 85 SEK (8.75 USD)/sample. If the farm owner collects milk from their own cows, the cost is estimated at 25 SEK (2.57 USD)/cow and collection of a BTM sample at 100 SEK (10.29 USD)/sample. The cost of a veterinarian taking blood samples is estimated at 75 SEK (7.72 USD)/animal.

Data Management

Herd-level data on health and production variables were retrieved from the DHI database (Växa Sverige, 2019) for the period September 1, 2018 to August 31, 2019. Data regarding mortality, culling rates, reproductive performance, and veterinary-reported clinical

diseases were calculated as cases per 100 animals at risk during the withdrawal period (1 yr). Herd size was calculated as the average number of cows (both lactating and dry) over the 12-mo period. Milk production was calculated as the mean production per cow in kilograms of ECM for the 12-mo period. Bulk tank milk SCC in thousands of cells per milliliter was calculated as the arithmetic mean of 12 monthly measurements. Breed was classified into 4 categories on herd level, with the main breed constituting more than 80% of the total number of cows.

Individual cow data on health and production were retrieved from the DHI database (Växa Sverige, 2019) for the period September 1, 2018, to February 28, 2022, including registrations for all cows in the herd during this time. Cow-level data consisted of lineage, calving events, milk production, registered veterinary diagnoses and treatments, registered reasons for culling, and mortality. The breed of the cow was categorized as Swedish Red (**SR**), Swedish Holstein (**SH**), or other breeds. If both parents were SR or SH, the cow was categorized as the same breed as their parents. Crosses or other breeds were categorized as other breeds. The following variables using calving dates were created: (1) from calving date to date of next calving, (2) calving date to date of removal from herd, or (3) calving date + 458 d (15 mo) if there was no later calving. These data were then used to determine the cow's calving number (1–9). The calving number was then connected to the test result in the relevant lactation period. If a cow had 2 ELISA antibody tests in the same lactation, the cow was registered as positive if at least 1 test was positive. Milk production was aggregated in kilograms of milk for 305 d in lactation, that is, the sum of each of the ~30-d lactation periods from calving to 305 d after calving. The 30-d lactation period was calculated for each monthly test milking day; milk yield of the test day was multiplied with 15 d before and 15 d after test milking, the sum gives kilograms of milk in 1 milk yield period. Registered veterinary treatments were grouped into udder diseases, hoof and leg diseases, and other diseases. The treatments were matched by date with that of the calving number of the cow for the statistical analysis. Removal of cows from the herd, culling, or death at farm was studied and matched with the cows that had test results.

In total, 2,338 samples from 1,860 cows were collected. There were some losses to follow-up for various reasons, for instance, 6 of the 35 herds were not affiliated with the DHI database (Växa Sverige, 2019) and therefore lacked information on individual cow registrations. In addition, some test results could not be included due to an incomplete 305-d lactation period, or the samples collected after the data withdrawal period ended (February 28, 2022). The final dataset used in the statistical analysis regarding milk yield contained 1,116 observations from 1,053 cows in 29 herds, and 63 cows (5.6%) had antibody test results in 2 lactations. In the models for culling or death at farm, 3 herds had incomplete data, resulting in 1,784 samples from 1,620 cows in 32 herds, and 156 cows (9.6%) had antibody test results in 2 or 3 lactations.

Statistical Analysis

To analyze the significance of the patterns of herd-level antibody prevalence in different age groups (Figure 2a), we undertook a Cochrane-Armitage test of trend of proportions. We categorized the sample 1 prevalence as <25%, 25% to 74%, and >75%, and evaluated the trend for herds with 4 antibody prevalence estimates in the 2 cow-level groups. For further investigation, we used a Fisher's exact test to examine if the proportion of increasing/decreasing prevalence varies between initial prevalence.

The statistical analysis assessed if the *M. bovis* antibody status (0 or 1) in cows was predictive of milk yield in 305-d lactation, culling, or death at farm, in 3 different models. The main explanatory variable was cow antibody test results in the lactation period in all 3 models. The objective of the analyses was to estimate population average associations between cow antibody status and milk yield, culling (0 or 1), or death at farm (0 or 1). Milk yield was a continuous variable, and a mixed linear regression model was applied, accounting for both fixed effects and random effects. The fixed effects represented the overall effect of the predictors on the outcome, while the random effects accounted for variations between herds. A generalized estimating equations (**GEE**) model (Liang and Zeger, 1986) with binomial distribution, logit link function, and exchangeable correlations structure between cows within herds was used for culling or death at farm. The models were adjusted for the biologically plausible confounders breed and calving number. Calving numbers were divided into 3 levels, first calving, second to third calving, and fourth calving or more. Breed was divided in SR, SH, and other. Initial models were created, with herd as cluster and milk yield (305 d), culling, or death as outcomes, and the predictor cow ELISA test result (negative/positive). Model evaluation was assessed with graphical inspection of the Pearson residuals and normal probability plots, checking the data for outliers and influential herds. The random effect was evaluated by plotting the random intercepts for each herd looking at herd-level predictions and likelihood ratio test. All statistical analyses were performed using Stata (release 15.1; StataCorp LP, College Station, TX).

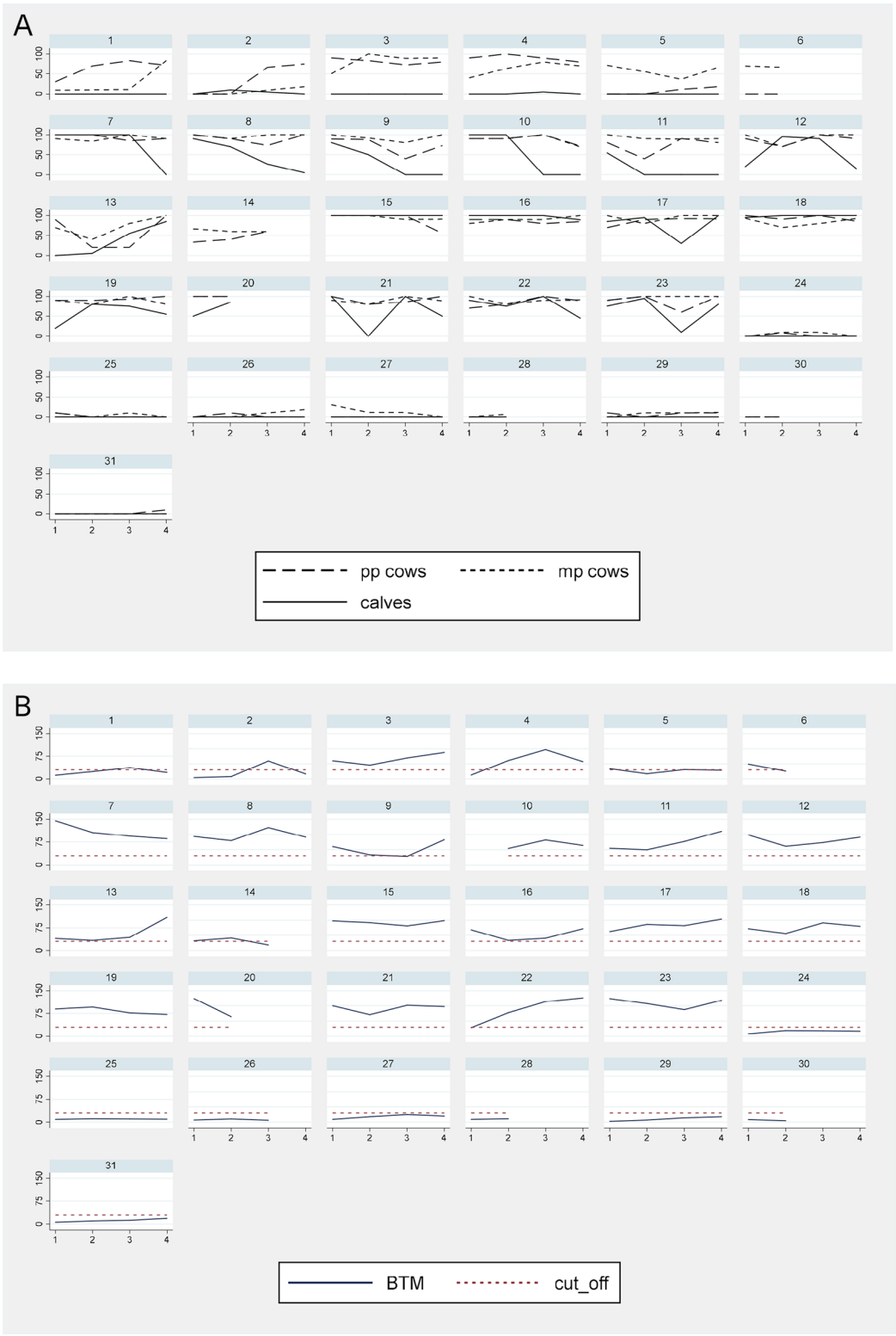

Figure 2. (A) Graphs by herd, within-group prevalence of antibodies to *Mycoplasma bovis* in the sampled cattle, primiparous cows (PP cows), multiparous cows (MP cows), and calves. The x-axis shows the number of the herd visit (sampling), and the y-axis shows the percentage of positive animals of the sampled animals in the herd. The average time interval between herd visits was 184 d (interquartile range [IQR] 170–210 d). (B) Graphs by herd, sample/positive (S/P%) in bulk tank milk (BTM) and cutoff level (S/P% ≥ 30%) for a positive ELISA test result (cutoff). The y-axis shows the S/P% (0%–150%), and the x-axis shows the number of the herd visit (sampling). The average time interval between herd visits was 184 d (IQR 170–210 d).

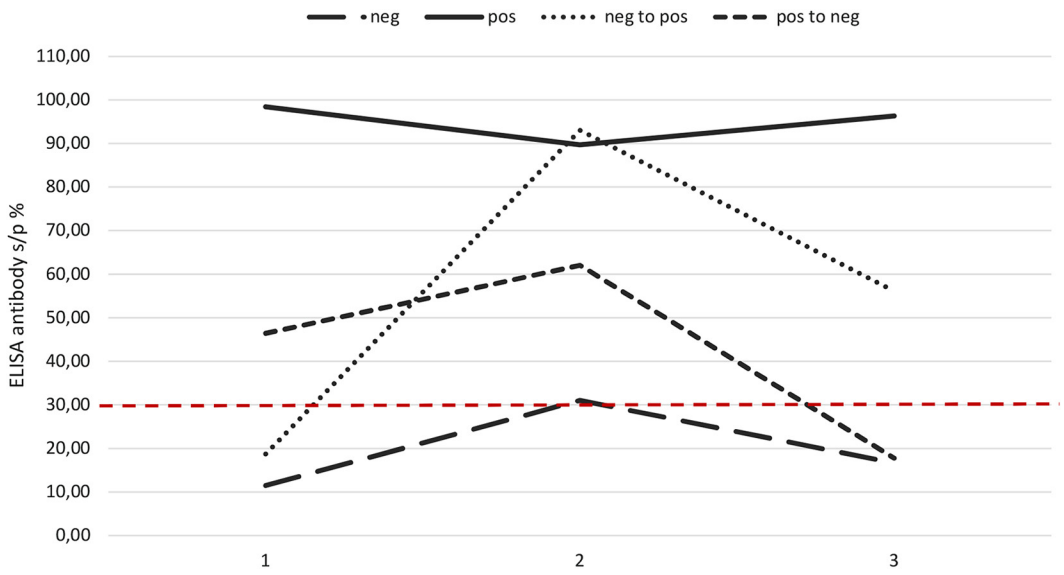

Figure 3. The average of the ELISA antibody sample/positive (S/P%) for cows with 3 samples with S/P% on y-axis and herd visits at x-axis. There are 4 different groups defined by the test result at the first and third herd visit. The number of cows in each group were negative (neg) = 12, positive (pos) = 57, neg to pos = 6, and pos to neg = 4. Cutoff level for a positive test result (S/P% ≥ 30%) is indicated by the horizontal dotted line. BTM = bulk tank milk.

RESULTS

Herd Characteristics

Median herd size and milk production were 150 cows (range 32–741) and 10,846 kg of milk per cow (range 8,973–12,625 kg), respectively. The distribution of breeds, on a herd level, was SH 43%, SR 11%, mixed SH and SR 26%, other breeds 3%, and unknown 17%. Culling incidence due to any reason, including mortality, in this 12-mo period, was a median 40% (range 34%–44%). The median incidence of veterinary-treated cases per 100 animals at risk per year for hoof and leg diseases and clinical mastitis were 2% (range 1%–4%) and 8% (range 4%–10%), respectively.

Each herd was visited and sampled between 1 and 4 times, and the median interval between samplings was 184 d (IQR 170–210 d). Twenty-seven herds completed 4 samplings, while 4 herds were sampled 2 times and the remaining 4 herds 1 time (Supplemental Table S1). There was a total of 120 sampling occasions, that is, herd visits during the study period and in total 2,338 samples from 1,860 cows, ~20% (n = 384) were sampled more than 1 time and 4% (n = 79) had 3 or more samples. The number of cows sampled at each herd visit was 19 to 21 in 87% (n = 104) of the visits, 17 to 18 samples in 5% (n = 6), 22 to 23 samples in 4% (n = 5), and 10 samples or less in 4% (n = 5). For PP and MP cows, 84% (n = 98) and 88% (n = 102) of the herd visits, respectively, included 8 to 12 samples (range 3–16). The parity of the cows was known for 117 visits for PP and 116 visits for MP cows. There were 115 herd visits with samples from calves, in total, 2,223 calves were sampled and in over 90% of the visits 20 calves (range 6–22) were sampled.

Overall Patterns of M. Bovis Antibody Status Interpreted at the Herd Level

For the herds with 4 samplings (n = 26), a stable pattern over time at cow level was observed in 18 herds; 5 of them had 0% to 25% positive cows at all sampling occasions, and 13 herds were positive for *M. bovis* antibodies in 75% to 100% of the cows throughout the study. The test for trend in proportions was not significant for these herds ($P > 0.05$) and there was no statistical evidence of increasing or decreasing prevalence compared with initial prevalence (Fisher's exact test $P > 0.05$). Four herds were sampled only 1 time, in which 2 were negative and 2 were positive both in calves and cows (Supplemental Table S1). The within-group prevalence of test-positive results at each sampling occasion was calculated for calves, PP, and MP cows (Figure 2a). There were 13 herds with variable patterns: 2 herds had positive PP and negative MP cows (Figure 2a, no. 1 and 2), and 2 herds had the opposite pattern with negative PP and positive MP cows (Figure 2a, no. 5 and 6). In addition, 2 herds had negative calves along with a high prevalence of positive cows and positive BTM (Figure 2a, no. 3 and 4). In 5 herds, the calves transitioned from positive to negative, while the cows remained positive (Figure 2a, no. 7–11).

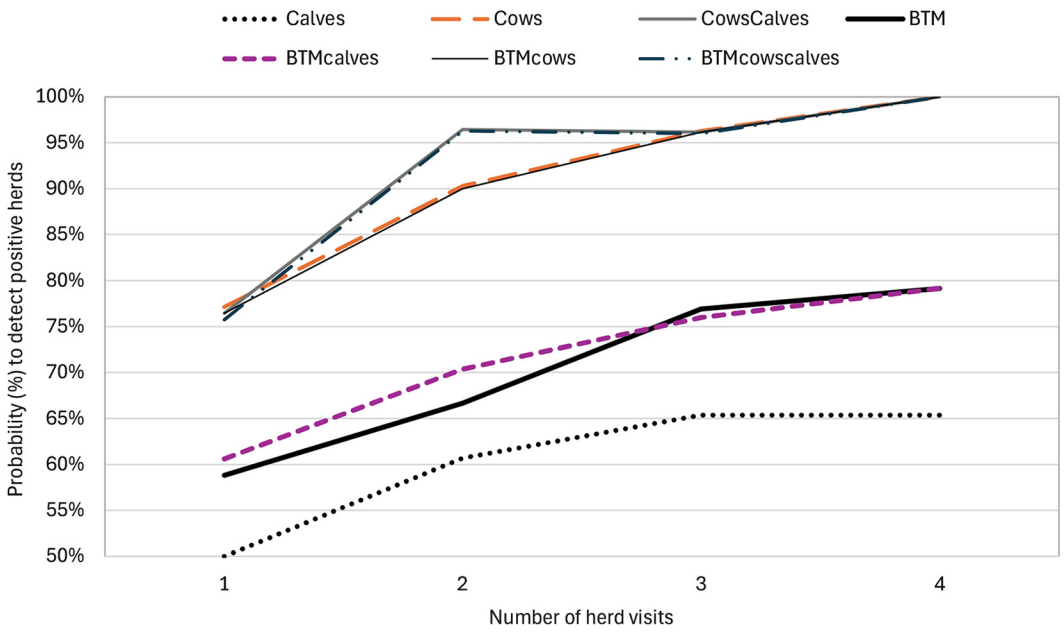

Figure 4. Probability (%) to detect positive herds by 7 different test strategies: calves only, cows only, cows and calves, BTM only, BTM and calves, BTM and cows, and BTM and cows and calves, at 1, 2, 3, or 4 herd visits. BTM = bulk tank milk.

One herd had a seasonally fluctuating pattern among the calves, while the cows and BTM were positive throughout the study period (Figure 2a, no. 21). Furthermore, in 1 herd, the calves transitioned from negative to positive along with an increasing prevalence in the cow groups and an increasing BTM S/P% (Figure 2a, no. 13).

At each herd visit, a BTM sample was taken and analyzed with the IDvet ELISA. In total, there were 117 BTM samples collected at the 120 herd visits, 75 (64%) were ELISA positive and 42 (36%) were negative. The *M. bovis* antibody ELISA test results of BTM ranged from 3 to 145 S/P% (Figure 2b).

Cow Samples

The 79 cows with 3 or more samples had ~6 mo intervals between herd visits. Individual test results for cows with 3 samples were grouped based on the first and third test results: negative in both samples (n = 12), positive in both samples (n = 57), or change in status either negative to positive (n = 6) or positive to negative (n = 4). The average S/P% was calculated in each of the 4 groups (Figure 3).

Test Strategy and Costs

The results from the 7 test strategies showed that the test sensitivity increased with the number of sampling occasions, that is, herd visits (Figure 4). All strategies that included tests on individual cow samples gave a higher sensitivity (Fisher's exact test $P < 0.05$) compared with BTM only, calves only, or BTM and calves combined.

Associations Between Seropositivity and Milk Yield, Culling, or Death at Farm

In the mixed linear regression model, there was a significant association ($P < 0.001$) between the predictor *M. bovis* positive test result and milk yield. The decrease in milk yield for a cow with *M. bovis* antibodies was 404 kg milk during a 305-d lactation (1.3 L/d, a 3.7% decrease compared with median milk yield in the herds; Table 3). Two herds were excluded from the statistical analysis of milk production because of incomplete data in the DHI database. This resulted in 1,089 samples from 1,026 cows in 27 herds being used in the final model with milk yield as the outcome. In the GEE models with culling incidence and death at farm, 1,784 samples from 1,620 cows in 32 herds were included. There were no significant associations between cow antibody status and culling, or death at farm (Table 3).

DISCUSSION

We report novel information on 3 aspects of the epidemiology and control of *M. bovis* in initially test-positive Swedish dairy herds. First, we described the patterns of *M. bovis* antibody response that indicate that *M. bovis* is initially introduced into the cow group and may subsequently, but not in all cases, be transmitted to the calves.

Table 3. The results of mixed linear regression and generalized estimating equations (GEE); regression coefficients with 95% CI, SE, and *P*-value, evaluating cow-level variables associated with antibody status to *Mycoplasma bovis* measured in individual cow milk[1]

Outcome	Predictor of interest, level	Coefficient (95% CI)	SE	*P*-value	Model adjustment variable
Milk yield, 305-d lactation in kg of milk (continuous)	*M. bovis* status				
	0: negative	Ref[2]			Breed
	1: positive	−404.5 (−719.4 to −89.6)	160.7	0.012	Parity
Culling due to any reason (0: negative, 1: positive)	*M. bovis* status				
	0: negative	Ref[2]			Breed
	1: positive	0.22 (−0.10 to 0.55)	0.17	0.175	Parity
Death at farm (0: negative, 1: positive)	*M. bovis* status				
	0: negative	Ref[2]			Breed
	1: positive	0.37 (−0.31 to 1.06)	0.35	0.289	Parity

[1]The variables are analyzed in lactation periods, and the antibody test results are matched with cow registrations in the relevant lactation.
[2]Ref = referent.

Second, we estimated the association between *M. bovis* antibody status and individual cow milk production that informs farmers of the financial consequences of exposure to *M. bovis*. Third, we estimated the combined diagnostic sensitivity of different herd-level testing strategies that can inform farmers of their *M. bovis* status in a cost-effective way.

Most of the herds had a stable pattern of antibody status over time with either a very low or a high prevalence in all age groups. Herds with antibodies in BTM kept a high prevalence of seropositivity in the cow group throughout the entire study period, showing that *M. bovis* infection was likely widespread among the cows before the herd entered the study. Also in other studies, the antibody response has been reported to persist for several months to more than a year after infection, the longevity of antibodies in individual animals is, at present, not well documented (Hazelton et al., 2018b; Vähänikkilä et al., 2019; Penterman et al., 2022). The high seroprevalence being maintained over time could be due to a repeated boosting of antibody levels because of reinfection by circulating *M. bovis*. Most of the herds with an initially low seroprevalence, that is, a small number of positive PP cows and negative BTM, continued to have only a few seropositive cows during the study period. These herds could be positive due to heifers or cows with historical antibodies being introduced to the herd, or heifers encountering other herds at external contract rearing facilities. In both cases, it is unlikely that they have shed bacteria after entering the sampled herd. In a herd with only a few cows with *M. bovis* antibodies, the overall herd health, regarding veterinary treatments, culling incidence, and mortality, is less likely to be affected. In about one-third of the herds, there were other patterns observed, for example, only PP or only MP cows being antibody-positive, or positive cows and negative calves. A limitation when interpreting these results is that we do not know when *M. bovis* was introduced to the herds, and differences in herd characteristics, regarding health and production, can be associated with the time of infection with *M. bovis*. However, studies of herds with recent outbreaks have shown that clinical cases are most common immediately following the first outbreak and decrease rapidly within a few months (Vähänikkilä et al., 2019; Penterman et al., 2022). In a few herds, there was evidence of a recent introduction of *M. bovis* with a rise in antibody levels in many animals. Repeated monitoring of antibody levels in a herd is important to capture such changes.

The patterns of antibody status in different age groups varied over time. In 5 herds, the antibody levels in the calf group decreased below cutoff at the third or fourth sampling, but it is unknown whether these farms made any interventions to protect the calves. However, the general recommendations from veterinary advisors to farmers of *M. bovis* test-positive herds are to remove the calves from the cow stable and to feed the calves with either milk powder or pasteurized milk (Haapala et al., 2021). New calves were tested at each sampling, suggesting that these 5 herds may have prevented transmission to young calves, and possibly the active infection had declined also among older cattle. One farm, however, made a significant intervention by building a new calf stable, thereby separating the calves from the cows. Together with a biosecurity barrier in the new calf stable, shifting clothes and boots, this appeared to successfully prevent transmission from cows to calves. Other herds may already have good routines in place, such as feeding the calves milk powder and keeping them separate from the cows, which makes it possible to keep the calves

M. bovis free despite a high seroprevalence among the cows. However, in 1 herd with initially negative calves and positive cows, the calves became positive at the third sampling, highlighting that sufficient biosecurity routines are necessary in everyday work. In a Finnish study, it was demonstrated that during *M. bovis* disease outbreaks, the infection spread across all age groups (Vähänikkilä et al., 2019). The transmission of *M. bovis* can occur from cows to calves and vice versa, depending on housing conditions and management (Biesheuvel et al., 2024). The present study, however, confirms that in Swedish dairy herds, the infection most likely enters the cow group first, because several herds have positive cows and negative calves. In some herds, the infection was first detected among the PP cows. This supports the claim that the introduction of animals is a source of infection (Fèvre et al., 2006), when considering that animals brought into dairy herds are typically either pregnant or younger heifers (Hurri et al., 2025).

A seasonal effect among the calves was observed in 2 herds in which the calves born during the spring and summer were not infected with *M. bovis*. This is likely due to the lower infection pressure with fewer circulating pathogens and fewer contacts between calves and older cattle when most of the herd is kept outdoors on pasture. *Mycoplasma bovis* infections increase when other pathogens circulate among the calves (Vulikh et al., 2024). Once established, the *M. bovis* bacterium is difficult to eradicate from a herd, and this study demonstrates that BTM-positive herds continue to have high levels of antibodies in the milk for several years. Interventions can, however, protect the calves from *M. bovis* infection, and this is key to stop the chain of infection and prevent further spread of the disease (Haapala et al., 2021).

In this study, we confirm previous findings which have shown that adding samples from individual cows increases herd-level test sensitivity compared with sampling BTM only (Hurri et al., 2025). The IDvet ELISA has a high sensitivity across different age groups, estimated as 92.5%, with no significant difference observed between samples from calves and cows following a clinical outbreak (Veldhuis et al., 2023). Sampling BTM is straightforward and inexpensive compared with sampling individuals, and repeated testing of BTM will increase the probability of detecting positive herds, from 60% on a single test to almost 80% in the third test round. This can be compared with a 90% probability of finding infected herds already on the second test, when testing individual cows with a high SCC. The testing was carried out with 6 mo intervals in regions where *M. bovis* is present in dairy herds, which suggests that this testing strategy is suitable for a control program. An additional benefit of adding individual cow samples was an earlier detection of a positive herd with only moderately increased sampling costs, which would make this test strategy a useful surveillance tool. However, a more exact number of individual cows necessary for effective herd testing with adequate sensitivity requires further investigation.

One limitation of this study was the potentially inadequate sample size within each age group of animals. The sample size of 20 cows and 20 calves was determined feasible considering cost and workload at the herd visits, and in combination with risk-based sampling and repeated testing, this would give a high probability of finding positive animals. However, this may have reduced the sensitivity for detecting exposure, leading to misclassification of group prevalence and lower precision in prevalence estimates. Additionally, there were some losses to follow-up and instances of noncompliance with sampling protocols by a few herds. Such challenges—limited resources and noncompliance—are common in many longitudinal observational field studies.

Among the cows with 3 samples, a few transitioned from seropositive to negative, suggesting that these individuals have a low risk of shedding *M. bovis*, and that it is possible for individual animals to eliminate the infection. With strict internal biosecurity measures, *M. bovis* infection could eventually be cleared from the herd. In a Finnish study, 13 out of 19 dairy herds were classified as low risk after implementing interventions (Haapala et al., 2021). Repeated sampling of individual cows also showed that 90% (n = 57/61) of the antibody-positive cows remained positive for ~12 mo. This provides evidence that *M. bovis* antibodies can be detected by the IDvet ELISA for at least 12 mo, and high antibody levels could suggest that these cows were chronically infected or became reinfected during this period. Monitoring antibody levels over time in individual cows can enhance knowledge about the infection status, as decreasing or increasing antibody levels can potentially contribute to a plan for the sectioning of the cows in high- or low-risk animals. In a previous study, we showed that an increasing number of infected cows also increased the antibody levels in BTM (Hurri et al., 2025). This was also observed in this study, suggesting that if antibody levels in BTM are high, testing individual cows may not be necessary, at least not before sectioning or other interventions. However, the calf group should still be tested, as their status is independent of the cow group's status. It would be of value if *M. bovis* antibody levels in BTM could predict the proportion of infected cows in the herd, as is the case for persistent viral infections such as bovine leukemia virus, where testing BTM provides a relatively precise estimate of the number of infected cows within the herd (Nekouei et al., 2015). Further research work is needed to investigate this for *M. bovis* antibody levels in BTM.

Cows with a positive test for *M. bovis* antibodies had a significantly lower milk yield, which is in line with previous studies. Seropositive cows produced 4% less milk in a Californian study (Uhaa et al., 1990), and in a dairy herd in Estonia, the daily milk yield decreased by 3.0 kg (Timonen et al., 2017). Mastitis is associated with lower milk production and although *M. bovis* infected herds in Sweden, to the best of our knowledge, experience minimal cases of clinical mastitis due to *M. bovis*, this study suggests that *M. bovis* affects the cows subclinically. Decreased milk production associated with *M. bovis* test-positive status has economic consequences for farmers. Wilson et al. (1997) reported that intramammary infections caused by *Mycoplasma* spp. reduced the milk production in one lactation by 1,500 kg, and this was equivalent to a loss of $451.63. We estimated that a seropositive cow produced on average, during a 305-d lactation, 404 kg (3.7%) less milk compared with a seronegative cow, equal to a decrease in 1.3 L/d. In today's milk price (6.22 SEK/kg = €0.56/kg), this will correspond to a loss in income of €225 (2,513 SEK) per cow and lactation. The loss in income because of reduced milk production will also depend on the infection pressure. In this study, the number of antibody-positive cows was either low (10%) or high (90%), and the average herd size was 150 cows. Therefore, in a low-infected herd, the loss would be €3,369 (37,690 SEK) in 1 yr, and in a high-infected herd, €30,328 (339,240 SEK) in 1 yr. Moreover, herds with infected calves can suffer economic losses due to heifers leaving the herd earlier than anticipated (Petersen et al., 2019). Costs of *Mycoplasma* disease also include death and culling losses, because *M. bovis* disease is usually chronic and poorly responsive to treatment (Maunsell et al., 2011), and culling is recommended to control *M. bovis* mastitis (Pfützner and Sachse, 1996; Nicholas et al., 2016). In the present study, however, neither culling nor death at farm were associated with *M. bovis* antibody-positive status of the cow. This finding may be explained by the fact that clinical disease due to *M. bovis* in cows was rarely reported by the farmers in the study and because subclinical production loss may be unnoticed by the farmer.

CONCLUSIONS

In this longitudinal study, we have investigated patterns of *M. bovis* status over time, both at herd level and for individual animals, and provided new insights into the epidemiology of this infection. The *M. bovis* antibody status varied across different age groups, and the antibodies were more prevalent and stable in the cow group than in the calf group. The introduction of *M. bovis* to the herd most likely occurred among the cows. Further, several herds demonstrated that it is possible to prevent transmission from cows to calves within-herd. Testing individual cows in addition to BTM increased the probability of finding infected herds. In the prospects of a control program, fewer cows need to be tested with a risk-based sampling strategy, keeping the costs low. In this study, we also demonstrated that *M. bovis* infection could affect cows subclinically, thereby resulting in a reduction in milk yield. Thus, to control the spread of *M. bovis* infection is likely to contribute to reduced income loss for dairy herds in Sweden.

NOTES

The authors thank the farmers for participating in this study and being so kind and helpful at the herd visits. We also thank the veterinarians for their time and help with collecting the samples. Gabriella Hallbrink Ågren, Swedish University of Agriculture Sciences (Uppsala), is gratefully acknowledged for skillful laboratory work, and SLU for the travel grant (travel grants for internationalization, SLU.ua.2023.3.2.5-336) that enabled the study visit of the first author at the EpiCentre, Massey University, Palmerston North, New Zealand. The study was supported by grants from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Stockholm; FORMAS, grant no. 2018/00943 and 2020/02515). Supplemental material for this article is available at https://doi.org/10.6084/m9.figshare.29098043. The animal study was reviewed and approved by the regional ethics committee in Uppsala, approval number 5.8.18-02650/2019. Written informed consent was obtained from the farmers for participation in this study. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: BTM = bulk tank milk; GEE = generalized estimating equations; IQR = interquartile range; MP = multiparous cows; neg = negative; OD = optical density; pos = positive; PP = primiparous cows; ref = referent; SH = Swedish Holstein; SLU = Swedish University of Agricultural Sciences; S/P = sample/positive; SR = Swedish Red.

REFERENCES

Andersson, A. M., A. Aspán, H. J. Wisselink, B. Smid, A. Ridley, S. Pelkonen, T. Autio, K. T. Lauritsen, J. Kensø, P. Gaurivaud, and F. Tardy. 2019. A European inter-laboratory trial to evaluate the performance of three serological methods for diagnosis of *Mycoplasma bovis* infection in cattle using latent class analysis. BMC Vet. Res. 15:369. https://doi.org/10.1186/s12917-019-2117-0.

Biddle, M. K., L. K. Fox, and D. D. Hancock. 2003. Patterns of *Mycoplasma* shedding in the milk of dairy cows with intramammary *Mycoplasma* infection. J. Am. Vet. Med. Assoc. 223:1163–1166. https://doi.org/10.2460/javma.2003.223.1163.

Biesheuvel, M. M., C. Ward, P. Penterman, E. van Engelen, G. van Schaik, R. Deardon, and H. W. Barkema. 2024. Within-herd transmission of *Mycoplasma bovis* infections after initial detection in dairy cows. J. Dairy Sci.107516–529

Caswell, J. L., and M. Archambault. 2007. *Mycoplasma bovis* pneumonia in cattle. Anim. Health Res. Rev. 8:161–186. https://doi.org/10.1017/S1466252307001351.

Ericsson Unnerstad, H., K. Fungbrant, K. Persson Waller, and Y. Persson. 2012. *Mycoplasma bovis* hos kor och kalvar i Sverige. Svensk Vet. 13:7–20.

Fèvre, E. M., B. M. C. Bronsvoort, K. A. Hamilton, and S. Cleaveland. 2006. Animal movements and the spread of infectious diseases. Trends Microbiol. 14:125–131. https://doi.org/10.1016/j.tim.2006.01.004.

Haapala, V., N. Vähänikkilä, L. Kulkas, E. Tuunainen, T. Pohjanvirta, T. Autio, S. Pelkonen, T. Soveri, and H. Simojoki. 2021. *Mycoplasma bovis* infection in dairy herds-Risk factors and effect of control measures. J. Dairy Sci. 104:2254–2265. https://doi.org/10.3168/jds.2020-18814.

Hazelton, M. S., J. M. Morton, K. L. Bosward, P. A. Sheehy, A. M. Parker, C. J. Dwyer, P. G. Niven, and J. K. House. 2018a. Isolation of *Mycoplasma* spp. and serological responses in bulls prior to and following their introduction into *Mycoplasma bovis*-infected dairy herds. J. Dairy Sci. 101:7412–7424. https://doi.org/10.3168/jds.2018-14457.

Hazelton, M. S., P. A. Sheehy, K. L. Bosward, A. M. Parker, J. M. Morton, C. J. Dwyer, P. G. Niven, and J. K. House. 2018b. Short communication: Shedding of *Mycoplasma bovis* and antibody responses in cows recently diagnosed with clinical infection. J. Dairy Sci. 101:584–589. https://doi.org/10.3168/jds.2017-13512.

Hazelton, M. S., J. M. Morton, A. M. Parker, K. L. Bosward, P. A. Sheehy, C. J. Dwyer, P. G. Niven, and J. K. House. 2020a. *Mycoplasma bovis* and other *Mollicutes* in replacement dairy heifers from *Mycoplasma bovis*-infected and uninfected herds: A 2-year longitudinal study. J. Dairy Sci. 103:11844–11856. https://doi.org/10.3168/jds.2020-18921.

Hazelton, M. S., J. M. Morton, A. M. Parker, P. A. Sheehy, K. L. Bosward, J. Malmo, and J. K. House. 2020b. Whole dairy herd sampling to detect subclinical intramammary *Mycoplasma bovis* infection after clinical mastitis outbreaks. Vet. Microbiol. 244:108662. https://doi.org/10.1016/j.vetmic.2020.108662.

Hurri, E., K. Alvåsen, S. Widgren, A. Ohlson, A. Aspán, K. Pedersen, and M. Tråvén. 2025. A longitudinal study of the dynamics of *Mycoplasma bovis* antibody status in primiparous cows and bulk tank milk in Swedish dairy herds. J. Dairy Sci. 108:845–855. https://doi.org/10.3168/jds.2024-25304.

Hurri, E., A. Ohlson, Å. Lundberg, A. Aspán, K. Pedersen, and M. Tråvén. 2022. Herd-level prevalence of *Mycoplasma bovis* in Swedish dairy herds determined by antibody ELISA and PCR on bulk tank milk and herd characteristics associated with seropositivity. J. Dairy Sci. 105:7764–7772. https://doi.org/10.3168/jds.2021-21390.

Liang, K. Y., and S. L. Zeger. 1986. Longitudinal data analysis using generalized linear models. Biometrika 73:13–22. https://doi.org/10.1093/biomet/73.1.13.

Marquetoux, N., M. Vignes, A. Burroughs, E. Sumner, K. Sawford, and G. Jones. 2023. Evaluation of the accuracy of the IDvet serological test for *Mycoplasma bovis* infection in cattle using latent class analysis of paired serum ELISA and quantitative real-time PCR on tonsillar swabs sampled at slaughter. PLoS One 18:e0285598. https://doi.org/10.1371/journal.pone.0285598.

Maunsell, F. P., A. R. Woolums, D. Francoz, R. F. Rosenbusch, D. L. Step, D. J. Wilson, and E. D. Janzen. 2011. *Mycoplasma bovis* infections in cattle. J. Vet. Intern. Med. 25:772–783. https://doi.org/10.1111/j.1939-1676.2011.0750.x.

Ministry of Primary Industries. 2023. Bulk tank milk screening for *Mycoplasma bovis* (*M. bovis*). New Zealand. Accessed Nov. 13, 2024. https://www.ospri.co.nz/farmers-and-livestock-owners/mycoplasma-bovis/how-mycoplasma-bovis-surveillance-works/bulk-tank-milk-screening-for-mycoplasma-bovis/.

Nekouei, O., H. Stryhn, J. VanLeeuwen, D. Kelton, P. Hanna, and G. Keefe. 2015. Predicting within-herd prevalence of infection with bovine leukemia virus using bulk-tank milk antibody levels. Prev. Vet. Med. 122:53–60. https://doi.org/10.1016/j.prevetmed.2015.10.009.

Nicholas, R. A., and R. D. Ayling. 2003. *Mycoplasma bovis*: Disease, diagnosis, and control. Res. Vet. Sci. 74:105–112. https://doi.org/10.1016/S0034-5288(02)00155-8.

Nicholas, R. A., L. K. Fox, and I. Lysnyansky. 2016. *Mycoplasma* mastitis in cattle: To cull or not to cull. Vet. J. 216:142–147. https://doi.org/10.1016/j.tvjl.2016.08.001.

Parker, A. M., P. A. Sheehy, M. S. Hazelton, K. L. Bosward, and J. K. House. 2018. A review of *Mycoplasma* diagnostics in cattle. J. Vet. Intern. Med. 32:1241–1252. https://doi.org/10.1111/jvim.15135.

Penterman, P. M., M. Holzhauer, E. van Engelen, D. Smits, and A. G. J. Velthuis. 2022. Dynamics of *Mycoplasma bovis* in Dutch dairy herds during acute clinical outbreaks. Vet. J. 283–284:105841. https://doi.org/10.1016/j.tvjl.2022.105841.

Petersen, M. B., A. K. Ersbøll, K. Krogh, and L. R. Nielsen. 2019. Increased incidence rate of undesired early heifer departure in *Mycoplasma bovis*-antibody positive Danish dairy cattle herds. Prev. Vet. Med. 166:86–92. https://doi.org/10.1016/j.prevetmed.2019.03.013.

Petersen, M. B., J. Pedersen, D. L. Holm, M. Denwood, and L. R. Nielsen. 2018. A longitudinal observational study of the dynamics of *Mycoplasma bovis* antibodies in naturally exposed and diseased dairy cows. J. Dairy Sci. 101:7383–7396. https://doi.org/10.3168/jds.2017-14340.

Petersen, M. B., L. Pedersen, L. M. Pedersen, and L. R. Nielsen. 2020. Field experience of antibody testing against *Mycoplasma bovis* in adult cows in commercial Danish dairy cattle herds. Pathogens 9:637. https://doi.org/10.3390/pathogens9080637.

Pfützner, H., and K. Sachse. 1996. *Mycoplasma bovis* as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. Rev. Sci. Tech. 15:1477–1494. https://doi.org/10.20506/rst.15.4.987.

Sachse, K., H. S. Salam, R. Diller, E. Schubert, B. Hoffmann, and H. Hotzel. 2010. Use of a novel real-time PCR technique to monitor and quantitate *Mycoplasma bovis* infection in cattle herds with mastitis and respiratory disease. Vet. J. 186:299–303. https://doi.org/10.1016/j.tvjl.2009.10.008.

Timonen, A. A. E., J. Katholm, A. Petersen, K. Mõtus, and P. Kalmus. 2017. Within-herd prevalence of intramammary infection caused by *Mycoplasma bovis* and associations between cow udder health, milk yield, and composition. J. Dairy Sci. 100:6554–6561. https://doi.org/10.3168/jds.2016-12267.

Uhaa, I. J., H. P. Riemann, M. C. Thurmond, and C. E. Franti. 1990. A cross-sectional study of bluetongue virus and *Mycoplasma bovis* infections in dairy cattle: I. The association between a positive antibody response and production efficiency. Vet. Res. Commun. 14:461–470. https://doi.org/10.1007/BF00367058.

Vähänikkilä, N., T. Pohjanvirta, V. Haapala, H. Simojoki, T. Soveri, G. F. Browning, S. Pelkonen, N. K. Wawegama, and T. Autio. 2019. Characterisation of the course of *Mycoplasma bovis* infection in naturally infected dairy herds. Vet. Microbiol. 231:107–115. https://doi.org/10.1016/j.vetmic.2019.03.007.

Växa Sverige. 2019. Cattle Health Statistics 2019. Accessed Nov. 13, 2024. www.vxa.se.

Veldhuis, A., M. Aalberts, P. Penterman, P. Wever, and G. van Schaik. 2023. Bayesian diagnostic test evaluation and true prevalence estimation of *Mycoplasma bovis* in dairy herds. Prev. Vet. Med. 216:105946. https://doi.org/10.1016/j.prevetmed.2023.105946.

Vulikh, K., D. Burrows, J. Perez-Casal, S. Tabatabaei, and J. L. Caswell. 2024. Effects of inflammatory stimuli on the development of *Mycoplasma bovis* pneumonia in experimentally challenged calves. Vet. Microbiol. 297:110203. https://doi.org/10.1016/j.vetmic.2024.110203.

Wawegama, N. K., G. F. Browning, A. Kanci, M. S. Marenda, and P. F. Markham. 2014. Development of a recombinant protein-based enzyme-linked immunosorbent assay for diagnosis of *Mycoplasma bovis* infection in cattle. Clin. Vaccine Immunol. 21:196–202. https://doi.org/10.1128/CVI.00670-13.

Wilson, D. J., R. N. Gonzalez, and H. H. Das. 1997. Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects on somatic cell count and milk production. J. Dairy Sci. 80:2592–2598. https://doi.org/10.3168/jds.S0022-0302(97)76215-5.

Zhang, R., X. Han, Y. Chen, R. Mustafa, J. Qi, X. Chen, C. Hu, H. Chen, and A. Guo. 2014. Attenuated *Mycoplasma bovis* strains provide protection against virulent infection in calves. Vaccine 32:3107–3114. https://doi.org/10.1016/j.vaccine.2013.12.004.

ORCIDS

E. Hurri, https://orcid.org/0000-0002-3240-7409
C. W. R. Compton, https://orcid.org/0000-0002-0821-1537
K. Alvåsen, https://orcid.org/0000-0001-7321-7030
M. Tråvén https://orcid.org/0000-0002-0936-0542