J. Dairy Sci. 105
https://doi.org/10.3168/jds.2021-21390

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

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ABSTRACT

Mycoplasma bovis is an important pathogen causing pneumonia, mastitis, and arthritis in cattle, leading to reduced animal welfare and economic losses worldwide. In this cross-sectional study, we investigated the prevalence of *M. bovis* in bulk tank milk (BTM) and herd characteristics associated with a positive antibody test result in Swedish dairy herds. Bulk tank milk samples from all Swedish dairy herds (n = 3,144) were collected and analyzed with ID Screen antibody ELISA and PCR. Information on herd characteristics was collected from the national Dairy Herd Improvement database. To identify herd characteristics associated with the presence of antibodies in BTM, logistic regression was used in 4 different models. The apparent herd-level prevalence of *M. bovis* infection based on antibodies in BTM was 4.8%, with large regional differences ranging from 0 to 20%. None of the BTM samples was positive by PCR. All the antibody-positive herds were situated in the south of Sweden. The logistic regression model showed that larger herds had higher odds of detectable antibodies in BTM (herd size >120 cows, odds ratio = 8.8). An association was also found between antibodies in BTM and both a higher late calf mortality (2–6 mo) and a higher young stock mortality (6–15 mo). This study showed a clear regional difference in the apparent prevalence of *M. bovis* infection based on antibodies. The relatively low prevalence of *M. bovis* in Sweden is a strong motivator for the cattle industry to take steps to prevent further spread of the infection. It is essential that the *M. bovis* status of free herds be known, and the regional differences shown in this study suggest that testing is highly recommended when live cattle from high-prevalence areas are being introduced into herds. We do not recommend using PCR on BTM to detect infected herds, owing to the low detection frequency in this study.

Key words: bulk tank milk, ELISA, herd size, *Mycoplasma bovis*

INTRODUCTION

Mycoplasma bovis is an emerging pathogen that causes severe disease in cattle in many countries (Maunsell et al., 2011), most often pneumonia, mastitis, arthritis, and middle ear infection (Nicholas and Ayling, 2003). During the past 10 years, *M. bovis* has been detected in new areas: first in Sweden in 2011 (Ericsson Unnerstad et al., 2012), in Finland in 2012 (Vähänikkilä et al., 2019), and in New Zealand in 2017 (Dudek et al., 2020). This bacterium is naturally resistant to penicillin and infections often fail to respond to broad-spectrum antibiotics, resulting in chronic disease that compromises animal welfare and causes great economic loss for the cattle industry (Nicholas and Ayling, 2003). Some risk factors that have been identified for dairy herds with *M. bovis* include large herd size (Thomas et al., 1981); purchase of animals (Burnens et al., 1999); forestripping, high milk production, and within herd movements (Aebi et al., 2015); and use of a breeding bull and lack of calving pens (Gille et al., 2018). Elimination of *M. bovis* from infected herds is believed to be difficult or even impossible, although raising calves separately from older animals has been suggested (Pfützner and Sachse, 1996; Aebi et al., 2015). In extreme situations, culling all the animals may be done (Pothmann et al., 2015). In cases of *M. bovis* mastitis, the recommendations are to separate and cull infected animals instead of attempting treatment (Fox et al., 2005; Nicholas et al., 2016).

Preventing infection both on the herd and the animal levels is the key to success. Given the lack of an effective commercial vaccine (Perez-Casal et al., 2017), the core of prevention needs to be based on herd diagnostics and the identification and elimination of epidemiological

Received October 7, 2021.
Accepted April 27, 2022.
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risk factors for *M. bovis* infection. Attempts to control the disease are ongoing in both Finland (voluntary control program) and New Zealand (eradication) (Dudek et al., 2020).

Diagnosing *M. bovis* is a challenge both on the individual animal level and the herd level. Culturing, which is laborious, time-consuming, and costly, used to be the only available method for *M. bovis* detection (Sachse et al., 1993). With the development of commercial antibody ELISA and PCR tests, screening of animals has become feasible, and the presence of *M. bovis* in a herd can more easily be detected (Cai et al., 2005; Wawegama et al., 2014; Andersson et al., 2019). The advantages of antibody tests are that they are simple, inexpensive, and rapid; in addition, they also detect previous infection, which is useful in identifying herds for further investigation. A newly developed antibody ELISA, the ID Screen (IDvet), has a diagnostic sensitivity and specificity of 95.7 and 100%, respectively, according to the manufacturer. The studies underlying these figures, however, have not been published. In an interlaboratory comparison between BIO K302 ELISA (Bio-X Diagnostics) and the ID Screen ELISA, applying a latent class analysis, the sensitivity and specificity were high for the ID Screen—sensitivity, 93.5%, (95% posterior credibility interval 0.898–0.965) and specificity, 98.6%, (95% posterior credibility interval 0.976–0.994)—and statistically significantly lower for the BIO K302 (Andersson et al., 2019). An earlier study using an in-house version of the ID Screen method showed similar results (sensitivity, 94.3%; specificity, 94.4%) (Wawegama et al., 2016). In a study by Petersen et al. (2020), the ID Screen was evaluated under field conditions, and the observed correlation between serum and milk values showed that milk samples could replace serum samples for antibody measurement. Individual cows showed high levels of antibodies, which is encouraging for use of the test on bulk tank milk (**BTM**) samples as well (Petersen et al., 2020). A positive correlation between *M. bovis* antibodies in BTM and in sera from individual cows was shown in a limited number of herds (Vähänikkilä et al., 2019), using an in-house ELISA with the same antigen (Wawegama et al., 2014). To our knowledge, our study is the first time that the ID Screen ELISA has been used in a nation-wide study of *M. bovis* prevalence in dairy herds. Multiple types of PCR are being used in different laboratories, including both in-house and commercial ones, with the majority being real-time PCR (Wisselink et al., 2019). The PCR testing has a high analytical sensitivity and can detect bacterial loads between 10 and 240 cfu/mL in milk (Parker et al., 2018); however, PCR analysis depends on active shedding of *M. bovis* in the milk. This is a challenge because *M. bovis* is shed intermittently (Biddle et al., 2003), and in many cases, milk from infected cows is not necessarily in the BTM at the time of sampling. The result of a single PCR analysis could thus lead to an underestimation of the herd prevalence (Petersen et al., 2016).

One objective of this study was to investigate the prevalence of *M. bovis* in Swedish dairy herds by determining the presence of specific antibodies and *M. bovis* DNA in BTM samples. A second objective was to study herd characteristics and herd location associated with a positive BTM sample using herd health and production data from the DHI database and mapping of the herds.

MATERIALS AND METHODS

This study involved no invasive procedures or handling of animals out of normal routine. Ethical approval or consent to participate was therefore not required.

Study Population and Sampling

Bulk tank milk from 3,144 Swedish dairy herds was collected at the milk testing laboratory (Eurofins Steins Laboratory, Jönköping, Sweden) in November 2019, in conjunction with routine milk quality analysis. Sweden had a total of 3,174 dairy herds at this time, which implies that 99.1% of all dairy herds were included. The samples were collected in 10-mL test tubes containing 1.5 mg of the preservative agent bronopol (2-bromo-2-nitropropane-1,3-diol). The samples were stored at −20°C until analysis.

Laboratory Analysis

All BTM samples (n = 3,144) were analyzed using real-time PCR (PathoProof Mastitis Major 4, Thermo Fisher Scientific), according to the manufacturer's instructions, at Eurofins Steins Laboratory. The cutoff for positive samples was set to cycle threshold <40 according to the manufacturer's instructions.

The samples were sent by postal service to the Swedish University of Agricultural Sciences (**SLU**), Uppsala. Out of all samples, 75 went missing during handling and transportation; these samples were not specific to any region. The remaining 3,069 samples were analyzed for antibodies to *M. bovis* with ID Screen indirect ELISA at the Department of Clinical Sciences, SLU, according to the manufacturer's instructions. The relative amount of antibodies in the samples was calculated as [sample optical density (OD) − negative control OD]/[positive control OD − negative control OD] × 100 (**S/P%**). The BTM samples were analyzed with the overnight incubation protocol and the cutoff for a positive sample

Table 1. Summary of the continuous variables showing the number of herds (n) and the median and interquartile range (IQR) for herds with no antibodies against *Mycoplasma bovis* (negative) and herds with *M. bovis* antibodies (positive) based on a total of 1,583 herds[1]

	Negative			Positive			
Item	n	Median	IQR	n	Median	IQR	*P*-value[2]
Milk production[3]	1,442	10,181	9,151–11,043	97	10,289	9,447–11,326	0.10
BTM SCC[4]	1,485	245	192–303	98	255.5	215–305	0.17
Calving interval,[5] mo	1,461	13.3	12.7–14.1	98	13.2	12.7–14.0	0.83
Age at first calving,[6] d	1,451	841	794–914	97	849.5	793–900	0.69
Cows at >70 d calving to first insemination,[7] %	1,446	21.7	15.0–32.4	97	20.0	14.2–28.1	0.23
Cows at >120 d calving to final insemination,[8] %	1,462	6.6	4.6–8.7	98	7.0	5.3–9.1	0.052
Culling for any reason including cow mortality[9]	1,462	33.6	27.4–40.8	98	36.1	29.9–42.9	0.036

[1]Each herd was tested with a *M. bovis* ELISA on a single bulk tank milk (BTM) sample.

[2]*P*-value from Student's *t*-test.

[3]Mean production per cow (kg of ECM).

[4]Measured as 1,000 cells/mL, arithmetic mean of 12 monthly measurements.

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

[6]Mean age at first calving for heifers giving birth during the 12-mo period.

[7]Number of cows in the 12-mo study period with an interval between calving and first insemination of >70 d divided by the mean number of cows with >70 d passed since calving (i.e., including cows calving within 70 d before the study period), not including cows calving within 70 d before the end of the study period.

[8]Number of cows in the 12-mo study period with an interval between calving and final insemination of >120 d divided by the mean number of cows with >120 d passed since calving (i.e., including cows calving within 120 d before the study period), not including cows calving within 120 d before the end of the study period.

[9]Cases per 100 animals at risk.

was set to S/P% $\geq$ 30% as suggested by the manufacturer.

Data Collection

Herd-level data on health variables were retrieved from the DHI database (Växa Sverige) for the period of November 1, 2018, to October 31, 2019 (i.e., the 12-mo period ending just before the BTM sampling). Data on herd size were additionally retrieved from Växa Sverige for the same time period. Of the herds with both PCR and antibody analysis, 2,258 (74%) were affiliated with the DHI program and 3,011 (98%) had data regarding herd size. For the statistical analysis of herd characteristics, only herds from regions with at least 1 positive herd were included. The total number of herds was 2,103, among which 1,583 (75%) were affiliated with the DHI program and 2,059 (98%) had data regarding herd size. Observations were missing for some variables. For each variable, a single value was obtained for each herd, presented in Tables 1 and 2. Data regarding mortality, culling rates, reproductive performance, and veterinary-treated clinical diseases were calculated as cases per 100 animals at risk. Herd size was calculated as the average number of cows (both lactating and dry) over the 12-mo study period. Milk production was calculated as the mean production per cow (ECM, kg) for the 12 mo that data were collected. 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 at the herd level, with the main breed consisting of more than 80% of the cows. Distribution of breed at the herd level was Swedish Holstein (**SH**), 31%; Swedish Red (**SR**), 7%; mixed SH and SR, 26%; and other breeds, 36%. The 2 main dairy cow breeds in Sweden are SR and SH.

Statistical Analysis

All variables were checked for outliers and unreasonable values. For the variables "% cows with >70 d between calving and first insemination" and "% cows with veterinary-treated diseases," 28 and 1 observations, respectively, were omitted because of values of >100%. The variables that were not linearly related to the logit of the outcome were either transformed to achieve normal distribution (BTM SCC and "% cows with >70 d from calving to first insemination" were log-transformed; milk production and calving interval were transformed by cubic function), categorized into equally sized groups, or dichotomized by median or by 0 and >0, according to Tables 1 and 2.

The statistical analysis assessed the effects of herd size on herd-level *M. bovis* antibody status (negative/positive) and whether herd-level *M. bovis* antibody status was predictive of the various herd health outcomes.

Table 2. Number of herds in levels of categorical variables, categorized into equally sized groups or dichotomized by median or by 0 and >0, showing herds with no antibodies to *Mycoplasma bovis* (negative) and herds with *M. bovis* antibodies (positive) based on bulk tank milk samples

Variable	Level	Negative (%)	Positive (%)	*P*-value[1]
Herd size (cows)	1: <40	443 (21.6)	9 (6.3)	<0.001
	2: 40–69	573 (28.0)	26 (18.1)	
	3: 70–119	475 (23.2)	38 (26.4)	
	4: >120	557 (27.2)	71 (49.3)	
Calf mortality (0–24 h),[2] %	0: 0–4.99	747 (51.1)	48 (49.0)	0.80
	1: >4.99	714 (48.9)	50 (51.0)	
Early calf mortality (1–60 d)[2,3]	0: 0–1.66	746 (51.1)	34 (34.7)	0.002
	1: >1.66	715 (48.9)	64 (65.3)	
Late calf mortality (2–6 mo)[2,3]	0: 0	988 (67.7)	42 (42.9)	<0.001
	1: >0	472 (32.3)	56 (57.1)	
Young stock mortality (6–15 mo)[2,3]	0: 0	1,021 (70.1)	45 (45.9)	<0.001
	1: >0	435 (29.9)	53 (54.1)	
Culling first parity cows early lactation (0–90 d)[3]	0: 0–1.6	723 (49.5)	29 (29.6)	0.001
	1: >1.6	737 (50.5)	69 (70.4)	
Culling due to udder diseases[3]	0: 0–6.9	732 (50.1)	49 (50.0)	0.99
	1: >6.9	730 (49.9)	49 (50.0)	
Culling due to hoof and leg diseases[3]	0: 0–1.69	742 (50.7)	38 (38.8)	0.023
	1: >1.69	720 (49.3)	60 (61.2)	
Culling due to reproduction diseases[3]	0: 0–6.94	737 (50.4)	43 (43.9)	0.21
	1: >6.94	725 (49.6)	55 (56.1)	
Cow mortality[2,3]	0: 0–4.93	740 (50.6)	41 (41.8)	0.094
	1: >4.93	722 (49.4)	57 (58.2)	
All veterinary-treated diseases[3]	0: 0–16.4	734 (50.2)	53 (54.1)	0.91
	1: >16.4	727 (49.8)	45 (45.9)	
Veterinary-treated clinical mastitis[3]	0: 0–6.7	739 (50.5)	41 (41.8)	0.10
	1: >6.7	723 (49.5)	57 (58.2)	
Veterinary-treated hoof and leg diseases[3]	0: 0	645 (44.1)	29 (29.6)	0.006
	1: >0	817 (55.9)	69 (70.4)	
Heifers >17 mo not inseminated, %	0: 31.95	733 (50.1)	47 (48.0)	0.61
	1: >31.95	729 (49.9)	51 (52.0)	

[1]*P*-value from chi-squared test.

[2]Mortality includes death and euthanization.

[3]Cases per 100 animals at risk.

Each of the herd variables was first evaluated by chi-squared test (χ^2) for categorical variables and Student's *t*-test for the continuous ones. All variables with $P \leq 0.20$ were further analyzed in multivariable logistic or linear regression models, correcting for biologically plausible variables. A backward stepwise approach for model building was used, starting with a full model and at each step eliminating one variable at a time ($P \geq 0.05$) from the regression model to find a reduced model that best explained the data. At each step the variable with the highest *P*-value was removed, and when all remaining variables had a *P*-value ≤ 0.05 the regression model was final. After omitting a variable, previous omitted variables were tested again, and the model was re-examined, and the selection of variable was reviewed again. This was possible because we had a limited number of variables in the full model. The presence of confounding was assessed by examining the effect of each predictor variable on the coefficient of other variables in the model by adding and removing them into and out of the model and examining the change in the coefficients of the remaining model variables. A complete description of the variables included in each regression model is available in Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.19323563; Hurri et al., 2022). Model fit was assessed with Hosmer-Lemeshow goodness-of-fit test, and plots of Pearson residuals versus the predicted values were constructed and evaluated for outliers. All statistical analyses were performed using Stata (release 17.0; StataCorp LP).

RESULTS

Prevalence

In total, 147 of the herds (4.8%) tested antibody ELISA positive, and the positive BTM samples ranged from 30.6 to 172.8 S/P%. All the herds tested PCR negative. The true herd-level prevalence of *M. bovis* based on antibodies in BTM was estimated as 3.8% (95% CI 3.0–4.7%), using Epitools Epidemiological Calculators (Sergeant, 2018), based on the diagnostic sensitivity (93.5%) and specificity (98.6%) for the ID Screen antibody ELISA (Andersson et al., 2019). The

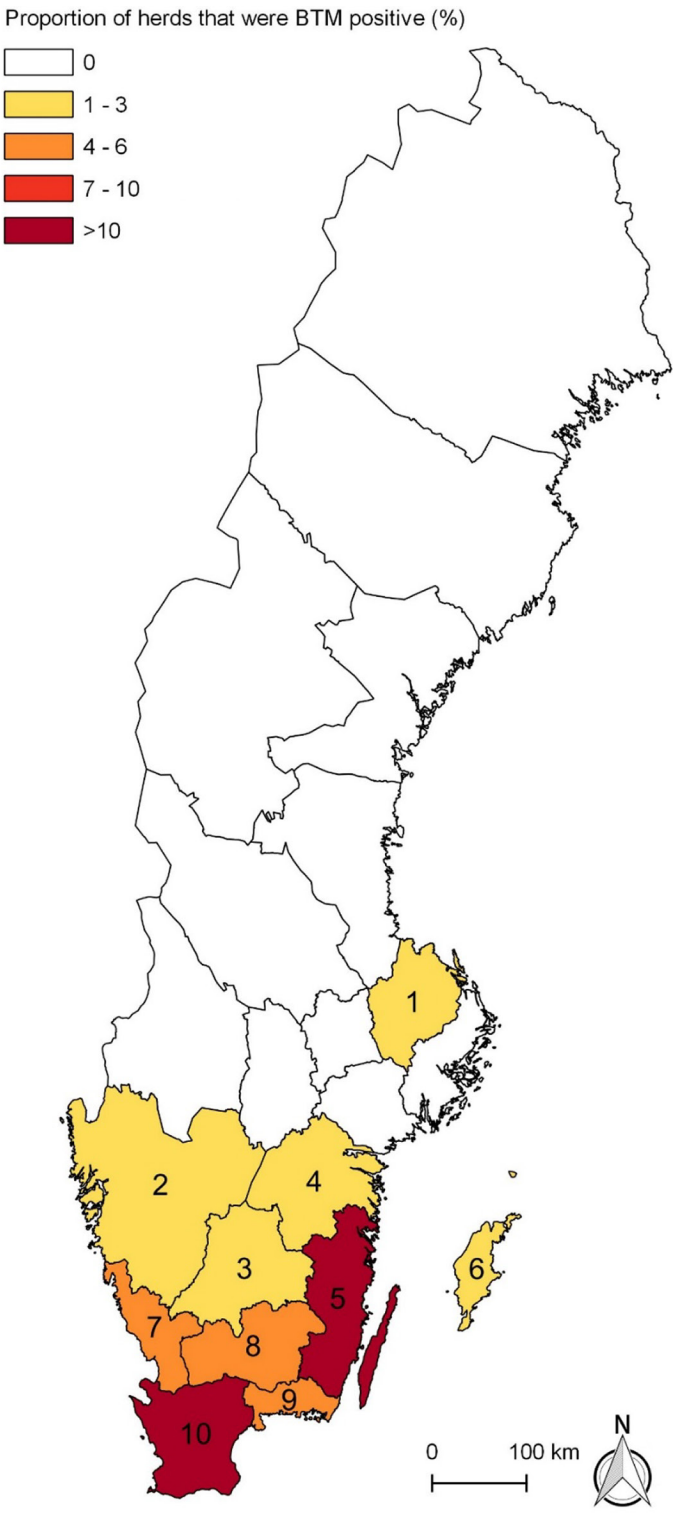

Figure 1. The proportion (%) of herds with antibodies to *Mycoplasma bovis* in bulk tank milk (BTM) for each of the 21 geographic regions in Sweden: 1 = Uppsala (1%, n = 1); 2 = Västra Götaland (3%, n = 16); 3 = Jönköping (3%, n = 10); 4 = Östergötland (3%, n = 7); 5 = Kalmar (13%, n = 41); 6 = Gotland (3%, n = 4); 7 = Halland (4%, n = 6); 8 = Kronoberg (5%, n = 6); 9 = Blekinge (6%, n = 3); 10 = Skåne (20%, n = 53).

apparent herd-level prevalence of *M. bovis* showed regional differences (0–20%), and almost two-thirds of the positive herds (n = 94) were situated in just 2 provinces in the south and southeast of Sweden (Skåne and Kalmar; Figure 1). The rest of the positive herds were located in the other 7 regions in the south, except for 1 positive herd (45.1 S/P%) in Uppsala in central Sweden. The proportion of herds that were BTM positive in each of the 21 regions in Sweden are shown in Figure 1. The term prevalence in this paper refers to herd-level prevalence.

Analysis of Herd Characteristics

Because none of the herds located in the middle and north of Sweden were antibody positive, with the exception of Uppsala, these regions were not included in the analyses of associations between herd characteristics and herd-level seropositivity. The region (isle) of Gotland had 4 positive herds, but these herds were not affiliated with the DHI program. The regions included in the analysis of associations between herd characteristics and herd-level seropositivity were Uppsala, Östergötland, Västra Götaland, Jönköping, Kronoberg, Kalmar, Blekinge, Halland, and Skåne (Figure 1). This corresponds to DHI data from approximately 69% of the *M. bovis* positive herds (98/143) and 76% of the negative ones (1,485/1,960). The DHI herd health variables had between 1,539 and 1,583 observations (Tables 1 and 2), the variation is due to missing values and the exclusion of unreasonable values. For the herd size analysis, Gotland was additionally included and herd size was available from 98% of the positive herds (144/147) and 99% of the negative herds (2,048/2,091) in the regions with at least 1 positive herd.

In the initial screening of association between the herd-level *M. bovis* antibody status (negative or positive) and herd characteristics, 13 out of the 22 variables had a *P*-value of $\leq$0.20, and the variable "culling due to reproduction diseases" had a *P*-value of 0.21 (Tables 1 and 2); these 14 variables were further assessed in the model-building procedure. Each of the 14 variables were tested in linear or logistic regression models correcting for the effect of breed, herd size, milk production, and region. Three variables remained statistically significantly related to antibody status, and 1 variable was borderline statistically significant. The results of these 4 models are presented in Table 3. In summary, larger herds had a higher risk of antibody positivity, and statistically significant associations were found between antibody positivity and having a mortality of more than 0% in older calves (age 2–6 mo) as well as in young stock (age 6–15 mo). Moreover, compared with antibody-negative herds, antibody-positive herds

Table 3. The results of linear and logistic regression models; regression coefficients (Coef.) with SE, *P*-value, odds ratios (OR), and 95% CI of OR, evaluating herd level variables associated with antibody status against *Mycoplasma bovis* measured in bulk tank milk

Outcome	Predictor of interest, level	Coef.	SE	*P*-value	OR (95% CI)	Model corrected for	Model type
M. bovis status (0: negative, 1: positive)	Herd size (cows)					Region	Logistic regression
	1: <40	Referent	0.39	0.12	1		
	2: 40–69	0.61	0.36	0.001	2.00 (0.92–4.37)		
	3: 70–119	1.22	0.35	<0.001	3.77 (1.78–7.97)		
	4: >120	1.54			8.82 (2.35–9.90)		
Calf mortality 2–6 mo (0: 0%; 1: >0%)	*M. bovis* status					Herd size[1]	Logistic regression
	0: negative	Referent			1		
	1: positive	0.60	0.23	0.012	1.83 (1.17–2.86)		
Young stock mortality 6–15 mo (0: 0%, 1: >0%)	*M. bovis* status					Herd size[1]	Logistic regression
	0: negative	Referent			1		
	1: positive	0.57	0.23	0.008	1.77 (1.13–2.77)		
Cows >120 d calving to final insemination[2] (continuous)	*M. bovis* status					Herd size[1] Milk production[1]	Linear regression
	0: negative	Referent					
	1: positive	0.66[3]	0.34	0.052	Not applicable		

[1]Categorized into 4 equal-sized groups.

[2]The percentage of all cows in the herd whose calving to final insemination interval was >120 d.

[3]The proportion of cows that were >120 d from calving to final insemination was 0.66 percentage units higher in herds that were positive for *M. bovis* antibody.

tended to have a higher incidence of cows with more than 120 d between calving and final insemination ($P = 0.052$).

DISCUSSION

In this cross-sectional study we aimed to determine the apparent prevalence of *M. bovis* in Swedish dairy herds using the ID Screen ELISA and to identify herd characteristics associated with a positive antibody test result. Bulk tank milk was sampled from all Swedish dairy herds (3,144). In the analysis of herd characteristics, we used data from the DHI database at Växa Sverige and only herds in regions with positive herds were included (1,583).

This study is the first to analyze antibodies to *M. bovis* in BTM on a national level in Sweden, and to our knowledge, it is the first time the ID Screen has been used in a national screening study. Analyzing BTM antibodies to *M. bovis* is a useful screening tool in the field because it is inexpensive and rapid and shows correlation with the antibody levels in serum of individual cows (Vähänikkilä et al., 2019). Analyzing antibodies in milk with the ID Screen ELISA is also supported by another study, showing good correlation between milk and serum in individual animals (Petersen et al., 2020). In our study, the apparent prevalence of *M. bovis* infection based on antibodies in BTM was 4.8% for the whole country, but with large regional differences ranging from 0 to 20%. The estimated true prevalence for the whole country (3.8%; 95% CI 3.0–4.7%), indicates that Sweden has few *M. bovis*–infected dairy herds. In contrast to PCR, antibody ELISA does not detect circulation of the bacterium but can detect relatively recent previous infections with *M. bovis* in a herd. Antibodies in individual cows have been detected up to 1.5 years after infection (Vähänikkilä et al., 2019), but the duration of antibody responses has not been thoroughly studied. Previous data on BTM antibody prevalence are available from studies in other European countries; for example, the BTM antibody prevalence was 7.1% in Danish herds (Nielsen et al., 2015) and 24.8% in Belgian herds (Gille et al., 2018). In those studies, the BIO K302 ELISA was used. However, the BIO K302 did not have a good correlation between milk and serum (Petersen et al., 2016, 2018). Further, in recent studies the ID Screen has shown a higher sensitivity than the BIO K302 (Andersson et al., 2019; Petersen et al., 2020). Thus, our results clearly show a lower prevalence of *M. bovis* in Swedish dairy herds in comparison with other European countries. A possible explanation for the lower prevalence is the lower cattle density in Sweden than in the other countries, resulting in slower spread of the disease. Further, Sweden was most probably free of *M. bovis* until around 2011, when a few cases were diagnosed in both fattening herds and dairy herds in the most southern region, Skåne (Ericsson Unnerstad et al., 2012). Because our results are based on a single BTM sample from each herd, they may be false-negative results for several reasons; for example, few cows with antibodies may have been present in the herd, antibodies may not have formed yet when the test was done, or infection was only circulating among calves and young stock (Petersen et al., 2016; Parker et al., 2017). As a

consequence, the true herd-level prevalence of *M. bovis* infection in Sweden might be higher than what was found in our study. Nevertheless, the results support for the use of a sensitive antibody ELISA test on BTM to monitor herd exposure. Analyzing repeated BTM samples for antibodies to *M. bovis* may convey a higher security in determining the infection status of herds.

In this study the samples were analyzed with both PCR and antibody ELISA, but no herds were PCR positive on BTM. An earlier national screening in Sweden in 2016 that used PCR analysis on BTM showed an apparent prevalence of 0.3% (n = 10) for *M. bovis* (Landin et al., 2019). The limit of detection for the PCR depends on the gene that is being amplified, and this information was not available for the PCR used in the current study (PathoProof Mastitis Major 4; Thermo Fisher Scientific) (Lönsjö 2020). No information is currently available regarding this PCR test performance against the reference standard of BTM culture (Bauman et al., 2018). The current study showed a low detection frequency using a single PCR on BTM, which might be due to a low within-herd prevalence of *M. bovis* and to the fact that *M. bovis* is shed intermittently in the milk (Petersen et al., 2016). Furthermore, milk from cows going through an active infection with *M. bovis* may not be included in the BTM. In addition, the possibility also exists that *M. bovis* antibodies have developed in response to diseases other than mastitis caused by *M. bovis,* such as respiratory infections or arthritis (Nicholas et al., 2002). Other studies have shown a similar pattern. In Belgium, the percentage of herds testing positive for *M. bovis* was 24.8% based on antibodies in BTM and 7.1% based on PCR analysis of BTM (Gille et al., 2018). In Australian herds, including 19 dairy herds with a history of *M. bovis* disease and 6 herds with no such cases, a much higher percentage of BTM samples were positive by antibody ELISA (39%) than by PCR (4%) (Parker et al., 2017). Altogether, our results support previous findings that PCR testing of BTM highly underestimates the *M. bovis* prevalence.

All herds in the north of Sweden tested negative for *M. bovis* in our study. The positive herds were situated in the south, with the highest apparent prevalence in Skåne (20%) and Kalmar (13%). Although the infection seems to have spread among dairy herds in the south during recent years, Sweden still has a favorable situation regarding *M. bovis* compared with many other countries. Few cases of *M. bovis* mastitis have been reported in Sweden, although underdiagnosis is possible because mastitis samples are not routinely analyzed for *M. bovis* and cows with subclinical mastitis might be culled without bacterial diagnosis. The higher prevalence in the south is probably due to the introduction of *M. bovis* in Sweden, the first cases being diagnosed in Skåne in 2011 (Ericsson Unnerstad et al., 2012). The spread of *M. bovis* in the south could also have been facilitated by the higher cattle density and larger herd size in these regions compared with the northern parts. The herds in the south of Sweden (regions 2–10, Figure 1) have an average of 105 cows per herd compared with the average for the whole country, which is 95 cows per herd. In Skåne the average herd size is 119 cows for herds registered in the DHI database (Swedish Board of Agriculture, 2020; Växa Sverige, 2020). Large herd size is a risk factor for *M. bovis*, probably connected to more introductions of animals and more direct or indirect contacts with other herds (Thomas et al., 1981; Fox et al., 2003). Our study showed a strong association between herd size and a positive BTM sample. Another study identified introduction of animals as a risk factor for the presence of *M. bovis* in a herd (Burnens et al., 1999). We did not have information about the herds' history of introduction of animals, but it is possible that many large herds have expanded relatively recently and therefore are more likely to have introduced cattle from other herds.

In this study we explored the associations between antibody status for *M. bovis* and a set of herd health variables. Two variables were associated with a positive BTM sample: late calf mortality and young stock mortality. *Mycoplasma bovis* infections in calves and young stock commonly present as pneumonia, otitis media, arthritis, or a combination of these disorders (Maunsell and Donovan, 2009). In many cases, the clinical disease becomes chronic and unresponsive to treatment, which leads to increased mortality. Our results show that *M. bovis* status was predictive of mortality in animals that were 2 to 15 mo old, but not those that were younger. A reason could be that pneumonia usually affects calves more than 2 wk of age. These animals might be treated several times, and therefore, chronic effects and mortality are seen in older animals (2–15 mo). This finding is also supported in a study by Petersen et al. (2019) in which undesired early departure of heifers >90 d of age was more common in *M. bovis* antibody-positive herds than in negative herds. In that study, however, early departure included premature culling of heifers, while we only had information on mortality (death and euthanization).

The current study has some limitations, aside from the diagnostics being done on BTM, as discussed above. We did not have information on many herd-related factors, and thus we could not control for them. In addition, we had no information on when *M. bovis* was introduced in the seropositive herds, the severity of the disease, and how it had developed over time in each herd before sampling.

Further research in *M. bovis*-infected herds is needed to determine the effect on herd health and mortality. Also, the increased risk for *M. bovis* antibody positivity as herd size increases, could imply a different contact pattern to other herds compared with that of smaller herds, which calls for further research. The possibility of reverse causality contributing to this association seems unlikely as farmers in Sweden are not able to increase the number of milking cows to compensate for some cows being in poor health because indoor housing systems limit the herd size. The study also showed a trend for a positive BTM sample being associated with an increasing percentage of cows with more than 120 d between calving and final insemination. This result could be a sign of increased subclinical disease due to *M. bovis* that affects fertility (Fox, 2012), but it could also be due to other herd factors. The effect on fertility would be interesting to investigate further in *M. bovis*–positive herds.

In Sweden, the most severe *M. bovis*–related disease has been seen in fattening herds, although calves in dairy herds also appear to suffer from *M. bovis* (Ericsson Unnerstad et al., 2012; Hurri et al., 2021). Substantial economic losses are connected to mortality of calves and young stock in dairy herds, and calculations show that the cost is 315 euro per case (heifers) for late calf mortality and 680 euro per case for young stock mortality in 2020 (Växa Sverige, animal welfare costs online tool, https://www.vxa.se). To reduce the costs of disease and secure animal welfare, the whole cattle sector in Sweden has now decided to come together to prevent *M. bovis* spread (Växa Sverige, 2021).

CONCLUSIONS

In this cross-sectional study, we found a higher prevalence of *M. bovis* in Sweden than what was previously known. The herds with an antibody-positive BTM sample were all situated in the south of Sweden, which correlates well with earlier studies and reports from the field. Analyzing BTM by PCR seems unsuitable, owing to the low detection frequency in this study. Analyzing antibodies can provide a more correct prevalence of *M. bovis* infection and be a useful tool to identify infected herds. Large herd size was identified as a risk factor for infection. The association between infection status, as measured by BTM antibody ELISA, and young stock and late calf mortality suggests that *M. bovis* infection affects animal health and welfare in Swedish dairy herds. The relatively low prevalence of *M. bovis* in Sweden is a strong motivation to minimize the spread of this disease and reduce the costs for farmers and the consequences on animal health and welfare.

ACKNOWLEDGMENTS

The authors thank Gabriella Hallbrink Ågren at the Department of Clinical Sciences, SLU, for performing the ELISA analyses, and Linda Svensson at the Department of Disease Control and Epidemiology, SVA, for supplying the map in the Results section. The study was supported by a grant from The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS, grant no. 2018/00943). Ethics approval and consent to participate were not applicable to this study. The datasets generated and analyzed in this study are available at the Swedish National Data Service (snd.gu.se), https://doi.org/10.5878/ysyt-bd21. The authors have not stated any conflicts of interest.

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