Effect of tuberculin skin testing on serological results against *Mycobacterium avium* ssp. *paratuberculosis* (MAP): Evidence of distinct effects in MAP-infected and noninfected cows

E. Nunney,[1]* ● M. Crotta,[1] ● S. van Winden,[1] ● K. Bond,[2] ● M. Green,[3] ● and J. Guitian[1] ●
[1]Veterinary Epidemiology, Economics and Public Health, Department of Pathobiology and Population Sciences, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA, United Kingdom
[2]National Milk Records Group, Fox Talbot House, Greenways Business Park, Bellinger Close, Chippenham, Wiltshire, SN15 1BN, United Kingdom
[3]The School of Veterinary Medicine and Science, University of Nottingham, Sutton Warwickshire, CV8 2TL Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, United Kingdom

ABSTRACT

Johne's disease and bovine tuberculosis are diseases of economic, public health, and animal welfare importance. The single intradermal cervical comparative tuberculin (SICCT) test, which is used to determine bovine tuberculosis status as part of eradication schemes in the United Kingdom and some other countries, has been reported to interfere with the results of the widely used ELISA to detect antibodies against *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in milk. Better understanding of the relationship between SICCT and MAP tests can improve management and control of Johne's disease. The aim of this study was to characterize the relationship between SICCT testing and milk ELISA performance and to assess whether the immunological response to the SICCT test is different for MAP-infected cows and noninfected cows. We used repeated MAP milk ELISA test results of a cohort of 805,561 cows in the United Kingdom between 2010 and 2018 that had milk ELISA tests within 90 d of SICCT testing to identify cows likely to be infected. We then assessed, separately, for cows deemed to be MAP-infected and noninfected, the association between MAP test results and proximity to SICCT testing by means of survival analysis and generalized additive mixed models. The results were used to quantify the effect SICCT testing may have on performance of milk ELISA tests conducted soon after SICCT testing. At high prevalence levels (20%) of MAP in the infected herd, overall accuracy of the milk ELISA is not reduced when testing occurs within 14 d from SICCT testing. Milk ELISA values of cows deemed to be infected were highest when MAP testing was closer in time to SICCT testing, suggesting the SICCT test enhances antibody response for MAP in infected cows. This corresponds to higher sensitivity of the MAP milk ELISA when testing within 30 d of the SICCT test. For cows deemed to be noninfected, the effect of previous SICCT testing was delayed compared with infected cows, with MAP milk ELISA values peaking at around 15 d post-SICCT testing. For both, MAP-infected and noninfected cows, interference from SICCT test diminished 30 d after SICCT testing, suggesting post 30 d to be the most appropriate time for evaluating the milk ELISA for MAP after SICCT testing. Our results provide strong evidence that the effect of the SICCT test on serological response against MAP is different for MAP-infected versus noninfected cows and that, as a result of this distinct effect, it is possible to improve interpretation of MAP milk ELISA test results (higher accuracy) by taking into consideration time since SICCT testing.
Key words: *Mycobacterium avium* ssp. *paratuberculosis*, bovine tuberculosis, milk, ELISA, SICCT testing

INTRODUCTION

Johne's disease (**JD**), caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), is a chronic wasting disease of ruminants responsible for considerable economic losses to the dairy sector worldwide (Ott et al., 1999; Garcia and Shalloo, 2015; Barratt et al., 2018). Furthermore, the public health impact of MAP exposure is a growing concern. There is increasing evidence of a causal association between MAP and Crohn's disease, and in recent years, MAP exposure has been found to be associated with other diseases such as multiple sclerosis (Sechi and Dow, 2015; Ekundayo et al., 2022). *Mycobacterium avium* ssp. *paratuberculosis* herd-level prevalence in the United Kingdom has been

estimated to range from 59 to 77% (Velasova et al., 2017). Infected cattle shed MAP in colostrum, milk, and feces at varying rates; those with clinical signs are at greatest risk of shedding. Before clinical signs develop, a long latent period is commonly observed, where transmission via these routes can still take place (Nielsen et al., 2002a). Calves are most susceptible to MAP, infection primarily taking place in first few weeks or months after calving (Windsor, 2010). It is assumed that calves are infected primarily via the fecal-oral route (Sweeney, 1996, 2011; Lombard, 2011; Rathnaiah et al., 2017; Fecteau, 2018). Transmission from the dam can also take place in utero, and the risk of MAP infection has been shown to be higher for calves born to MAP-infected dams even when those dams were subclinical and seronegative at the time of calving (Whittington and Windsor, 2009; Patterson et al., 2020). Therefore, early detection of infection, to reduce the risk of transmission to calves, is crucial for MAP control. However, detecting MAP poses a challenge due to poor sensitivity of diagnostic tests, especially in early stages of infection (Hanks et al., 2013; Li et al., 2017). The most commonly used diagnostic test is the ELISA for detection of antibodies in milk samples. Although fecal culture has a higher sensitivity, the ELISA is relatively low cost, more convenient, and faster (Nielsen et al., 2002b; Stabel et al., 2002). Among the tests that can be used for diagnosis of MAP infection, the IFN-γ assay, which measures the levels of the cytokine IFN-γ, has been found capable to detect MAP infection at early stages (Nielsen and Toft, 2009; Corneli et al., 2021). This test has also been found to be strongly affected by a previous single intradermal cervical comparative tuberculin (**SICCT**) test, with subclinical MAP-infected cows becoming more likely to test positive after SICCT testing (Coussens, 2004; Stabel et al., 2007; Roupie et al., 2018).

The mean sensitivity and specificity of the milk ELISA across all age groups, given the age distribution of cows enrolled in a UK JD screening program, has been estimated at 61.8 and 99.9% (Meyer et al., 2018). The relatively low sensitivity can be explained by the chronic nature of the disease and the resulting gradual increase in antibody levels as infection progresses, with fluctuations that may correspond to transient shedding of the bacteria (Nielsen, 2008). To overcome the limitations associated with relatively low and age-varying sensitivity and fluctuation in antibody levels, allocation of MAP infection status to cows for the purpose of farm-level decisions, frequently relies upon repeated testing. Currently in the United Kingdom, decisions on control commonly involve selective culling based on whether a cow is classified as "green," "yellow," or "red"

from repeated milk ELISA testing. The widely adopted HerdWise service, which is recognized by the Cattle Health Certification Scheme, defines cows as "red" when they test MAP positive from milk ELISA twice within 4 consecutive tests. "Green" cows are cows that have never tested positive, and the remaining cows are classified as "yellow" (Meyer et al., 2018; HerdWise, 2022).

Interpretation of MAP milk ELISA results is further complicated by cross-reactivity with other mycobacteria such as *Mycobacterium bovis*. *Mycobacterium bovis* is the primary causative agent of bovine tuberculosis (**bTB**) in the United Kingdom, but it should be noted that according to some regulations such as the current European Animal Health Law, bTB is caused by mycobacteria of the *Mycobacterium tuberculosis* complex, namely *Mycobacterium bovis*, *Mycobacterium caprae*, and *Mycobacterium tuberculosis* (EU, 2020). Bovine tuberculosis is subject to a statutory national eradication program in the United Kingdom, with annual testing being mandatory in high-risk areas (APHA, 2020). In the United Kingdom, screening for bTB involves the SICCT test where the purified protein derivatives (**PPD**) are extracted from *M. bovis* AN5 (bovine PPD) and *M. avium* D4ER (avian PPD), respectively (Tameni et al., 1998; Corneli et al., 2021). The avian PPD and bovine PPD are injected into the neck at 2 different sites and infection status is determined 72 h postintradermal injections (Monaghan et al., 1994; de la Rua-Domenech et al., 2006).

Several studies have examined the potential of infection with bTB (as opposed to SICCT testing and how the PPD affect the milk ELISA) affecting the specificity of the MAP milk ELISA test (Byrne et al., 2019; Picasso-Risso et al., 2019) as well as the reverse relationship: infection with MAP affecting the accuracy of diagnosing bTB (Seva et al., 2014; Roupie et al., 2018). Research on the effect the SICCT has on MAP milk ELISA diagnostic performance is more limited. An increase in MAP antibodies post SICCT has been shown (Varges et al., 2009; Kennedy et al., 2014); however, it is not clear whether this rise leads to an increase in sensitivity due to a potential anamnestic effect from the PPD in MAP-infected animals or due to antibody cross-reaction in MAP-noninfected animals or a mixture of both. The anamnestic effect (booster effect) has been well described in tuberculosis in both animals and humans (Costello et al., 1997; Palmer et al., 2006). An increase in the sensitivity of antibody tests associated with a rise in serum antibody responses after intradermal injection of tuberculin has been identified in tuberculous camelids, deer, and other species (Busch et al., 2017; TB hub, 2021). The HerdWise pro-

gram in the United Kingdom recommends that cows should not be tested for MAP during the 42-d period following SICCT testing (HerdWise, 2022); however, this recommendation is not always followed and there have been anecdotal reports that MAP testing soon after SICCT testing may help identifying MAP-infected cows. A recent study performed by Barden et al. (2020) showed an increase in the odds of a positive MAP test result when MAP milk ELISA testing occurs less than 30 d to SICCT testing. A similar result was also found by Bridges and van Winden (2021), who reported an increased risk of testing positive in the MAP milk ELISA, with a gradually increasing risk, peaking 57 to 70 d post SICCT testing to subsequently wain. In this study, we aim to further characterize the relationship between SICCT testing and MAP milk ELISA performance, including an assessment of whether the immunological response to the SICCT test is different for MAP-infected cows and noninfected cows. Our specific objectives were (1) to determine the effect of time since SICCT testing on MAP milk ELISA test values [sample-to-positive (**S/P**) ratios] for MAP-infected and noninfected cows and (2) to estimate the effect of testing interval between SICCT and MAP on the capacity of the MAP milk ELISA to correctly classify cows according to their MAP infection status.

MATERIALS AND METHODS

Source of Data and Data Management

This historical longitudinal study was based on MAP milk ELISA test data provided by the National Milk Records group (**NMR**) and National Bovine Data Centre consisting of 10,153,441 MAP test results for 1,697,828 individual animals carried out between January 1, 2010, and September 29, 2018 (62% provided by NMR and 38% from National Bovine Data Centre in terms of individual cows). This data set includes the vast majority of MAP tests carried out in the United Kingdom during that period. Both data providers tested milking cows (usually every 3 mo) for MAP antibodies by milk ELISA (IDEXX Paratuberculosis Screening Ab Test, IDEXX Laboratories, Westbrook, ME) and interpreted the results following the manufacturer's instructions: tests with a S/P ratio of 0.3 or above were considered positive. After removal of duplicates, animals with inconsistencies in date of birth or the identifier (i.e., ear tag), animals more than 20 yr old, and animals with first test less than 20 mo, 9,714,164 MAP tests from 1,617,659 animals were available for analysis. Of these, 1,369,722 animals were present in the data set with more than one MAP test.

This data set was integrated with information on SICCT test dates that were systematically recorded between January 1, 2012, and March 1, 2021, kindly provided by the United Kingdom's Animal Plant Health Agency (**APHA**). The SICCT test uses avian PPD and bovine PPD to elicit a delayed hypersensitivity response mediated by T cells (Monaghan et al., 1994). Single intradermal cervical comparative tuberculin testing intervals vary depending on the bTB infection status of the herd from every 2 mo in herds currently under bTB restrictions to 1- to 4-yr intervals in bTB free herds.

Merging of the 2 data sets (i.e., MAP test and SICCT test) by animal ear tag resulted in 7,909,639 records from 1,073,499 unique animals for which MAP milk ELISA tests and SICCT tests could be paired. Each MAP milk ELISA test was matched to the closest preceding SICCT test; this meant that cows could have the same SICCT test paired to multiple MAP test observations. Selection of cows with clear SICCT test results and observations within 90 d of SICCT testing made up the final data set for analysis; this consisted of 2,404,368 MAP test observations from 805,561 cows.

Case Definition

Classification as MAP-infected was based on the HerdWise program definition for determining cattle highly likely to be infected and shedding MAP in the feces (HerdWise, 2022). The HerdWise program is a screening program provided by NMR where animals are tested quarterly using milk recording samples. According to the program, a cow with 2 positive milk ELISA test results within 4 consecutive tests is classified as infected. For this study, the same definition is used. To avoid interference from cows classified as infected that could have been classified due to the SICCT testing under consideration, cows defined as infected from tests occurring within 90 d from SICCT testing were excluded from the analysis (14% of infected cows). The remaining cows that did not meet this criterion were deemed to be noninfected.

Statistical Methods

A total of 66,156 infected cows and 739,405 noninfected cows were present from 3,226 herds after applying the case definition. All statistical models were built separately for infected and noninfected cows. Given the large number of records available and to reduce computation time, one observation from each noninfected cow and infected cow was randomly selected. Statistical models were estimated using packages coxme (Ther-

neau, 2020) and mgcv (Wood, 2017) from R version 4.1.0 (R Core Team, 2020).

Relationship Between Time from SICCT to MAP Test and MAP Positive Test Result

Nested frailty models were used to assess the (linear) relationship between time since SICCT test and MAP positive test result, with the cohort beginning when the first MAP test was recorded for each cow and the event of interest being a positive MAP milk ELISA test result.

We analyzed only the first 90 d after SICCT testing [this period was selected after examining the results of the generalized additive mixed models (GAMM) described below]. The time interval between SICCT and MAP testing was categorized into 4 groups: 0 to 14 d, 15 to 28 d, 29 to 60 d, and 61 to 90 d. The 61 to 90 d group was used as the baseline group for analysis. Hazard ratios and their 95% confidence intervals (CI) were obtained as a measure of the hazard of a MAP positive result for each category, relative to the 61 to 90 d period. The model accounts for clustering of the data by including a random frailty effect (herd). The random effect term was assumed to follow Gaussian distribution. The models were assessed with age as a potential confounder, which was incorporated into the final models.

Nonlinear Relationship of Time from SICCT to MAP Test and MAP Optical Density Values

Generalized additive mixed models were applied to assess potential nonlinear relationships between time from SICCT testing to MAP test and MAP milk ELISA values (S/P ratio). Due to the skewed nature of the ELISA results, the GAMM model was fitted using a gamma logarithm link function. A gamma distribution requires strictly positive data (>0); 2% of the S/P ratios were zeros, so to fit a gamma distribution to the variable, 0.001 was added to all values. To model the group variability among herds, different random effect structures were tested, and models were compared using Akaike information criterion. The final model contained time in days between SICCT and MAP testing and age (in days) as continuous variables and herd fitted as a random slope; additionally, each variable had its own smooth term using thin plate regression splines. Cross validation was used to estimate the amount of smoothing. Each smooth function was plotted for infected and noninfected cows with 95% confidence bands. Model diagnostics included plotting of residuals versus fitted values to assess homogeneity of variance, and of residuals versus each covariate to investigate model misfit.

Impact of Time Since SICCT Testing on Ability of MAP Test to Classify Cows as Infected Versus Noninfected

Using the case definition as the reference (i.e., assuming that cows that meet the case definition are MAP infected), the mean sensitivity and specificity for the MAP milk ELISA were calculated for MAP tests carried out at each time interval from SICCT testing. One observation from each cow was randomly sampled and the process was repeated 1,000 times to find the mean sensitivity and specificity with 95% CI. This was done separately for the entire data set and for the data set excluding cows defined as infected within 90 d from SICCT testing.

Mean accuracy and positive and negative predictive values of the test were simulated using a binomial probability model. Accuracy referred to the proportion of tests that correctly identify the infection status of the cow. Sensitivity and specificity values were randomly sampled from the range of values obtained when including and excluding cows classified as infected within 90 d from SICCT testing.

The simulation was repeated 10,000 times to calculate mean accuracy and predictive values with 95% CI for hypothetical 200-cow herds at 5, 10, and 20% true prevalence of MAP infection, for each SICCT-MAP testing time interval.

RESULTS

Descriptive Statistics

A total of 805,561 cows were present in the data set, of which 66,156 (8%) cows were deemed infected at some point in their lives, based on the case definition. The average number of cows tested per herd was 250. Descriptive statistics are presented in Table 1.

Relationship Between Time from SICCT to MAP Test and MAP Positive Test Result

Results from the nested frailty models are shown in Table 2. The models give strong evidence of an association between SICCT to MAP test time and positive MAP milk ELISA result, for both infected and noninfected cows ($P < 0.001$ for both models). Among cows assumed to be infected, tests carried out within 14 d from SICCT testing were 1.36 times (95% CI: 1.25–1.49) more likely to yield a positive result compared

Table 1. Descriptive statistics by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) status[1]

Group	MAP infected	MAP noninfected
Number of positive tests [n (%)]	112,929 (47.2)	46,569 (2.2)
Number of cows	66,156	739,405
Lactation		
Median	4	4
IQR[2]	3–6	3–5
Number of MAP tests per cow		
Median	8	7
IQR	6–13	4–10
Lactation of first positive test (yr)		
Median	4	4
IQR	3–5	3–6
Lactation of classification as case (yr)		
Median	4	—
IQR	3–6	—
Time between SICCT and MAP tests in days (%)		
Median	62	62
IQR	50–72	50–72

[1]Data from 2,404,368 individual test results from 805,561 cows belonging to 3,226 herds, tested by MAP milk ELISA in the United Kingdom between February 6, 2012, and August 29, 2018, within 1 to 90 d from single intradermal cervical comparative tuberculin (SICCT) testing.
[2]IQR = interquartile range.

with tests carried out 61 to 90 d after SICCT testing. The hazard ratio for infected cows decreased as time between SICCT and MAP testing increased. The probability of a MAP test being positive was also affected by time from SICCT to MAP test among noninfected cows. For noninfected cows, the risk of a MAP positive test result was highest at 15 to 28 d post-SICCT testing, with the effect being stronger than among infected cows (hazard ratio: 3.35; 95% CI: 3.11–3.61). Infected and noninfected cows had similar hazard ratios for MAP testing 29 to 60 d post SICCT testing, both showing a reduced risk of a positive MAP milk ELISA

test result compared with the previous time periods (Table 2).

Nonlinear Relationship of Time from SICCT to MAP Test and MAP Optical Density Values

The results from the GAMM are shown in Figure 1. The plots show the estimated relationship between time from SICCT to JD testing and S/P ratios from JD ELISA tests. For infected cows, the S/P ratios decrease steadily as time between SICCT to JD test time increases, reaching a plateau when JD testing occurs around 50 d post-SICCT testing. The effect of SICCT testing on S/P ratios for noninfected cows is less prominent, delayed, and shorter compared with infected cows. Sample-to-positive ratios peak around 15 d post SICCT and level off around 35 d post SICCT testing. Model checking showed residuals were approximately normally distributed and there did not seem to be a pattern when the residual versus fitted values were plotted.

Impact of Time Since SICCT Testing on Ability of MAP Test to Classify Cows as Infected Versus Noninfected

The increase in S/P ratio of infected cows immediately after SICCT testing results in infected cows having a higher probability of positive MAP milk ELISA result the closer MAP testing occurs to SICCT testing (higher sensitivity) and therefore increasing the probability that a negative test reflects a truly uninfected animal by lowering the probability of a false-negative result. The pattern in sensitivity is the same when including or excluding the 14% of infected cows classified within 90 d from SICCT testing. The effect of SICCT testing on S/P ratio of noninfected cows is smaller and delayed, resulting in a change (reduction) in specificity, which

Table 2. Estimates of Cox models examining association between time since single intradermal cervical comparative tuberculin (SICCT) test and *Mycobacterium avium* ssp. *paratuberculosis* (MAP) positive milk ELISA results (sample-to-positive ratio $\geq$ 0.3)[1]

SICCT to MAP test time (d)	MAP infected		MAP noninfected	
	Hazard ratio (95% CI)	*P*-value[2]	Hazard ratio (95% CI)	*P*-value
61–90	1		1	
1–14	1.36 (1.25, 1.49)	<0.0001	3.13 (2.92, 3.36)	<0.0001
15–28	1.25 (1.14, 1.38)	<0.0001	3.35 (3.11, 3.61)	<0.0001
29–60	1.00 (0.97, 1.04)	0.65	1.15 (1.11, 1.20)	<0.0001

[1]Herd used as random effect term (data from 66,156 infected and 739,405 noninfected tested between 2012 and 2018).
[2]Wald test.

is lowest (0.95; 95% CI: 0.95–0.95) when MAP testing occurred 15 to 28 d post-SICCT testing (Table 3). This drop is also seen in the positive predictive value and overall accuracy during this period across the range of prevalences (Table 4). There is small variation in negative predictive values over the different time intervals from SICCT testing. At high prevalence levels (20%) of MAP in the infected herd, overall accuracy of the milk ELISA is not reduced when testing occurs within 14 d from SICCT testing. The milk ELISA accuracy is the same as for testing 60 d after SICCT testing.
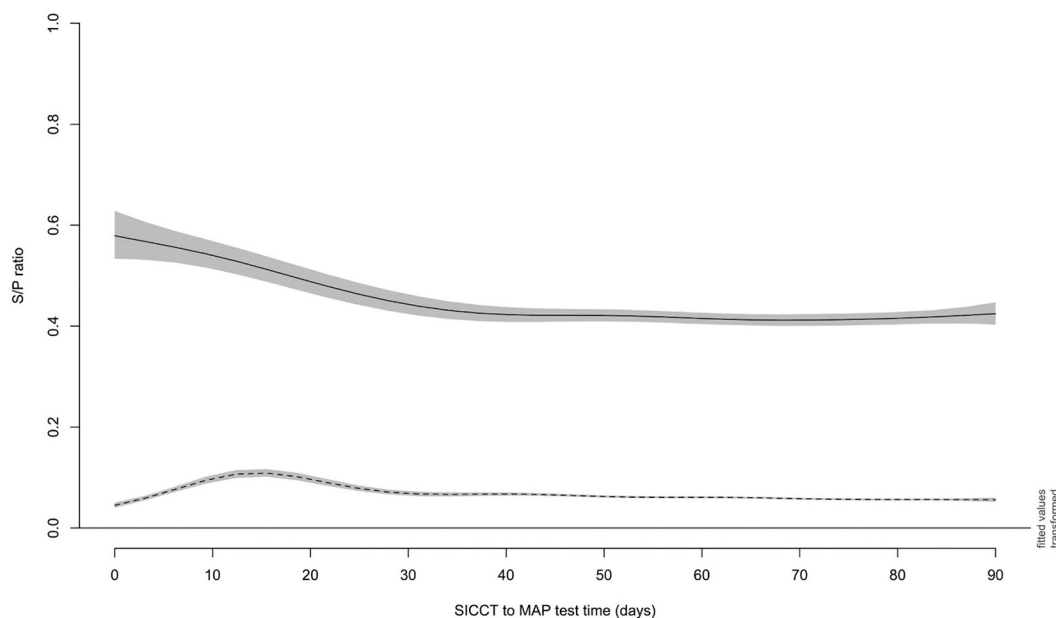
Table 3. Mean *Mycobacterium avium* ssp. *paratuberculosis* (MAP) milk ELISA sensitivity and specificity for tests carried out at different time intervals since single intradermal cervical comparative tuberculin (SICCT) testing with 95% CI[1]

SICCT to MAP test time (d)	All cows		Exclusion	
	Sensitivity	Specificity	Sensitivity	Specificity
1–14	0.65	0.96	0.40	0.96
15–28	0.62	0.95	0.37	0.95
29–60	0.54	0.98	0.34	0.98
61–90	0.53	0.98	0.32	0.98

[1]The results are presented when all infected cows are included in the analysis and when cows that were classified as infected within 90 d from SICCT testing were excluded.

DISCUSSION

Johne's disease and bTB are arguably 2 of the most important single-agent infectious diseases affecting the dairy industry in the United Kingdom and worldwide (Radia et al., 2013; Sibley, 2019; Whittington et al., 2019). Although a statutory program exists for bTB, JD control relies on identification of infected cows by means of repeated testing, due to the chronic and slow progressive nature of the disease and the limited performance of diagnostic tests during the long latent or preclinical phase of the disease. The economic importance of JD for the dairy industry and the reliance on repeated testing is reflected in the numbers of tests carried out: between 2010 and 2018, the 2 largest milk recording companies in the United Kingdom carried out 10,153,441 tests on milk samples from 1,697,828

individual cows belonging to 3,760 dairy herds. As for SICCT testing, between 2012 and 2019 in the United Kingdom, 5,238,938 tests were performed, with testing occurring up to every 60 d in bTB infected herds (Lilenbaum et al., 2007). In this context, characterizing the effect of the SICCT test on MAP test results and on the ability of MAP tests to classify cows as infected is of great importance as it can inform adaptation of testing regimens and interpretation.

An association between MAP milk ELISA results and the SICCT test is biologically plausible and has been proposed by several studies (Kennedy et al., 2014; May et al., 2016; Barden et al., 2020). Kennedy et al. (2014) provided evidence for a significant difference between ELISA values pre and post SICCT every 14 d up until

Figure 1. Generalized additive mixed model plots showing the relationship between *Mycobacterium avium* ssp. *paratuberculosis* (MAP) milk ELISA testing proximity to a single intradermal cervical comparative tuberculin (SICCT) test and MAP milk ELISA sample-to-positive (S/P) ratios for infected (solid line) and noninfected (dashed line) cows. The lines represent the S/P ratio from MAP milk ELISA test as a function of SICCT to MAP test time, with herd included as random slope. The shaded area represents the 95% confidence interval.

Table 4. *Mycobacterium avium* ssp. *paratuberculosis* (MAP) milk ELISA test accuracy, positive predictive value (PPV), and negative predictive value (NPV) when tests are carried out at different time intervals since SICCT testing in herds with different animal-level prevalence (Prev)[1]

SICCT to MAP test time (d)	Prev = 0.05			Prev = 0.10			Prev = 0.20		
	PPV	NPV	Accuracy	PPV	NPV	Accuracy	PPV	NPV	Accuracy
1–14	0.41 (0.20, 0.61)	0.97 (0.96, 0.99)	0.93 (0.91, 0.96)	0.59 (0.41, 0.77)	0.95 (0.92, 0.98)	0.92 (0.89, 0.95)	0.76 (0.64, 0.89)	0.89 (0.85, 0.93)	0.87 (0.83, 0.92)
15–28	0.34 (0.15, 0.53)	0.97 (0.95, 0.99)	0.93 (0.90, 0.95)	0.52 (0.35, 0.70)	0.94 (0.92, 0.97)	0.90 (0.87, 0.94)	0.71 (0.57, 0.84)	0.88 (0.84, 0.93)	0.86 (0.81, 0.90)
29–60	0.54 (0.28, 0.79)	0.97 (0.95, 0.99)	0.95 (0.93, 0.97)	0.71 (0.51, 0.90)	0.94 (0.91, 0.97)	0.93 (0.90, 0.95)	0.84 (0.73, 0.96)	0.88 (0.83, 0.92)	0.87 (0.83, 0.91)
61–90	0.53 (0.26, 0.79)	0.97 (0.95, 0.99)	0.95 (0.93, 0.97)	0.70 (0.50, 0.90)	0.94 (0.91, 0.97)	0.92 (0.90, 0.95)	0.84 (0.72, 0.96)	0.87 (0.83, 0.91)	0.87 (0.83, 0.91)

[1]The values in the table are the mean and 0.025 and 0.975 percentiles from 10,000 simulations randomly drawing sensitivity and specificity values from the range estimated in Table 3.

43 d after SICCT testing in a herd of 139 cows. Single intradermal cervical comparative tuberculin testing has also been shown to have a longer effect on serum ELISA samples compared with milk samples, and an increase in antibody titers in serum has been recorded up till 90 d post-SICCT testing (Varges et al., 2009; Kennedy et al., 2014). There has been no evidence, to our knowledge, whether the time span of this effect differs between MAP-infected and noninfected cows, which would have an effect on MAP test performance. A study conducted by May et al. (2016) on a herd of 240 cows examined the relationship separately for cows based on previous MAP test results; however, they did not have a large enough study population for meaningful comparison of cows with different MAP infection status ("red," "yellow"), and they did not find evidence of a difference between milk ELISA values pre and post SICCT testing. On a herd level, Bridges and van Winden (2021) found that the odds for finding an additional positive test is associated with a SICCT before the MAP milk ELISA test, and the study was not able to tell whether the effect was equal for positive and negative cows. A similar study by Barden et al. (2020) was able to examine over 20,000 cows from multiple herds. The effect of SICCT testing on the MAP milk ELISA testing was quantified through splitting SICCT test to MAP test time intervals into 4 groups (<30 d, 31–60 d, 61–90 d, >90 d). The groups showed an increase in odds of a positive MAP test result when testing less than 30 and 60 d post SICCT as well as an increased odds of a MAP positive test result if a large avian skin reaction was recorded at SICCT test before MAP testing. By examining how the SICCT leads to changes in the S/P ratios along the continuum of days post SICCT testing, we show that, in fact, MAP testing within 30 d after the SICCT drives S/P ratios to increase differently in MAP infected and noninfected animals.

The results of this study confirm the effect of SICCT on MAP milk ELISA results as seen by Barden et al. (2020) and, importantly, that this effect differs between MAP infected and noninfected cows. The results of the 2 analytical strategies adopted here, (1) survival analysis to explore a linear relationship between time since SICCT testing and MAP test results and (2) GAMM to allow for a more flexible, nonlinear relationship, are consistent and provide a detailed characterization of the relationship between SICCT and MAP test results over time. The probability of a previously unidentified infected cow testing positive to MAP is higher the earlier the MAP test is after SICCT testing independent of an anamnestic MAP positive test (cows defined as infected close to SICCT testing were excluded). This suggests a boosted immune response in MAP-infected

cattle (i.e., an anamnestic effect occurs following the injection of avian PPD) in animals infected with MAP. On the other hand, although in noninfected cows the S/P ratio also increases following SICCT testing, the response is small and delayed, suggesting a primary immune response to the PPD injected during the SICCT test. The response to the injection of the avian PPD in MAP-infected animals precedes the more delayed nonspecific response as a consequence of the simultaneous injection of both bovine and avian PPD. It seems unlikely that the same specific anamnestic effect seen in MAP infected cows would follow the caudal fold test or the single intradermal test, both of which use only bovine PPD.

As a result of the boosted immune response in MAP-infected animals, the main effect of SICCT testing on MAP test performance is through an increase in sensitivity, which results in a higher probability of MAP-infected cows being correctly classified as positive. The drop-in specificity is of a much smaller magnitude but of similar effect on overall accuracy for reasonable values of within herd prevalence (5 to 20%). Our results suggest that the improved classification of infected cows through MAP testing occurring in short succession after SICCT testing would not be offset by the misclassification of noninfected cows. The overall accuracy is similar in each time interval except for 15 to 28 d post SICCT testing and can be slightly higher or slightly lower when MAP tests are conducted soon after SICCT testing, depending on the within herd prevalence of infection. For high MAP prevalence values, which are not uncommon in UK herds (Carslake et al., 2011), testing for MAP immediately after SICCT testing would result in no decrease in overall accuracy.

A limitation of this study is the way we have classified cows as infected versus noninfected. Determining MAP status is challenging because there is no gold standard test (Laurin et al., 2017). The classification criteria used in this study can result in either false negatives (as the specificity of the milk ELISA is not 100% and we did not select noninfected cows from herds known to be MAP free) or false positives (as the sensitivity of the milk ELISA is not 100% even when cows are classified as infected despite some test results being negative). Therefore, we do not have complete certainty in the infectious status of cows we assumed to be infected or noninfected. However, the case definition used in this study has been estimated to yield a high specificity of 99.8% (Meyer et al., 2018). Therefore, it seems unlikely that noninfected cows have been misclassified as infected. However, there is potential for cows truly MAP infected to be misclassified as being noninfected due to the low sensitivity (66.8%) of the test (Meyer et al., 2018). We would expect their S/P ratio to be higher

and their behavior to be as that of the infected ones; in other words, if it has an effect, it would be toward underestimating the pattern we identified.

The ELISA test results are often unpredictable due to variation in the S/P ratios from repeated testing. For example, transient MAP shedders are more likely to have variable ELISA values compared with high shedding cows (Nielsen, 2008). Our study identifies SICCT testing as a contributing factor that could lead to a variable antibody pattern seen.

Previous research has also shown ELISA values to be affected by age, stage of lactation, and parity in MAP positive cows (Nielsen et al., 2002a; Toft et al., 2005; Meyer et al., 2018). Studies have also reported how infection with bTB can result in cross-reactive immune response to MAP, therefore affecting the sensitivity and specificity of the MAP serum ELISA test (Picasso-Rissso et al., 2019). It is unlikely that bTB infection has affected the MAP milk ELISA test results, due to the low prevalence in the United Kingdom and animals with a positive SICCT test being deemed reactors and culled (APHA, 2018). In addition, we restricted our study population to only examine cows with clear SICCT test results, as well as only examine cows classified as MAP infected outside of the 90-d post SICCT testing. After exclusion, our findings still support that SICCT testing induces distinct changes in the MAP milk ELISA results of infected and noninfected cows.

CONCLUSIONS

Single intradermal cervical comparative tuberculin testing affects the serological response of cows against MAP in different ways depending on whether the cow is MAP-infected or not. The probability of infected cows testing positive increases immediately after SICCT testing, whereas for MAP noninfected cows there is a slower response until an increase in milk ELISA S/P ratios is seen. This effect is at individual cow level and independent of the prevalence of infection in the herd. However, its practical implications in terms of the overall accuracy of MAP testing within a herd differ depending on the within herd prevalence. In herds with high prevalence of MAP infection, the overall accuracy of the milk ELISA is not reduced when testing occurs within close time proximity to SICCT testing. In high prevalence herds, testing for MAP antibodies soon after SICCT testing could be beneficial to improve test accuracy and to identify MAP-infected animals for control actions earlier than would otherwise be the case. To avoid interference from the SICCT test in both MAP infected and noninfected cows the milk ELISA should be evaluated more than 30 d post SICCT testing.

REFERENCES

Animal Plant Health Agency (APHA). 2018. Bovine Tuberculosis in England in 2018. Epidemiological Analysis of the 2018 Data and Historical Trends. Accessed May 8, 2020. www.gov.uk/government/uploads/system/uploads/attachment_data/file/413806/tb-pub-surveport-gb13.pdf.

Animal Plant Health Agency (APHA). 2020. 2020 Surveillance TB testing intervals policy (England). Accessed Sep. 21, 2020. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/911892/tb-testing-intervals-policy-england2020.pdf.

Barden, M., R. F. Smith, and H. M. Higgins. 2020. The interpretation of serial Johne's disease milk antibody results is affected by test characteristics, pattern of test results and parallel bovine tuberculosis testing. Prev. Vet. Med. 183:105134. https://doi.org/10.1016/j.prevetmed.2020.105134.

Barratt, A. S., M. H. Arnoult, B. V. Ahmadi, K. M. Rich, G. J. Gunn, and A. W. Stott. 2018. A framework for estimating society's economic welfare following the introduction of an animal disease: The case of Johne's disease. PLoS One 13:e0198436. https://doi.org/10.1371/journal.pone.0198436.

Bridges, N., and S. van Winden. 2021. The occurrence of *Mycobacterium avium* subspecies *paratuberculosis* positive milk antibody ELISA results in dairy cattle under varying time periods after skin testing for bovine tuberculosis. Animals (Basel) 11:1224. https://doi.org/10.3390/ani11051224.

Busch, F., F. Bannerman, S. Liggett, F. Griffin, J. Clarke, K. P. Lyashchenko, and S. Rhodes. 2017. Control of bovine tuberculosis in a farmed red deer herd in England. Vet. Rec. 180:68. https://doi.org/10.1136/vr.103930.

Byrne, A. W., J. Graham, G. Milne, M. Guelbenzu-Gonzalo, and S. Strain. 2019. Is there a relationship between bovine tuberculosis (bTB) herd breakdown risk and *Mycobacterium avium* subsp. *paratuberculosis* status? An investigation in bTB chronically and non-chronically infected herds. Front. Vet. Sci. 6:30. https://doi.org/10.3389/fvets.2019.00030.

Carslake, D., W. Grant, L. E. Green, J. Cave, J. Greaves, M. Keeling, J. McEldowney, H. Weldegebriel, and G. F. Medley. 2011. Endemic cattle diseases: Comparative epidemiology and governance. Philos. Trans. R. Soc. Lond. B Biol. Sci. 366:1975–1986. https://doi.org/10.1098/rstb.2010.0396.

Corneli, S., A. Di Paolo, N. Vitale, M. Torricelli, L. Petrucci, C. Sebastiani, M. Ciullo, L. Curcio, M. Biagetti, P. Papa, S. Costarelli, M. Cagiola, A. Dondo, and P. Mazzone. 2021. Early detection of *Mycobacterium avium* subsp. *paratuberculosis* infected cattle: Use of experimental Johnins and innovative interferon-gamma test interpretative criteria. Front. Vet. Sci. 8:638890. https://doi.org/10.3389/fvets.2021.638890.

Costello, E., P. F. O'Reilly, D. K. Yearsley, D. P. O'Grady, L. M. O'Reilly, J. D. Collins, M. L. Monaghan, and H. F. Bassett. 1997. A study of an enzyme-linked immunosorbent assay for the diagnosis of tuberculosis in cattle. Ir. Vet. J. 50:35–38.

Coussens, P. M. 2004. Model for immune responses to *Mycobacterium avium* subspecies *paratuberculosis* in cattle. Infect. Immun. 72:3089–3096. https://doi.org/10.1128/IAI.72.6.3089-3096.2004.

de la Rua-Domenech, R., A. T. Goodchild, H. M. Vordermeier, R. G. Hewinson, K. H. Christiansen, and R. S. Clifton-Hadley. 2006. Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. Res. Vet. Sci. 81:190–210. https://doi.org/10.1016/j.rvsc.2005.11.005.

Ekundayo, T. C., T. Olasehinde, A. Falade, M. Adewoyin, C. Iwu, B. Igere, and O. Ijabadeniyi. 2022. Systematic review and meta-analysis of *Mycobacterium avium* subsp. *paratuberculosis* as environmental trigger of multiple sclerosis. Mult. Scler. Relat. Disord. 59:103671. https://doi.org/10.1016/j.msard.2022.103671.

EU (European Union). 2020. 2020/689 of 17 December 2019 Supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as Regards Rules for Surveillance, Eradication Programmes, and Disease-Free Status for Certain Listed and Emerging Diseases. Accessed May 20, 2022. http://data.europa.eu/eli/reg_del/2020/689/oj.

Fecteau, M. E. 2018. Paratuberculosis in cattle. Vet. Clin. North Am. Food Anim. Pract. 34:209–222. https://doi.org/10.1016/j.cvfa.2017.10.011.

Garcia, A. B., and L. Shalloo. 2015. Invited review: The economic impact and control of paratuberculosis in cattle. J. Dairy Sci. 98:5019–5039. https://doi.org/10.3168/jds.2014-9241.

Hanks, J. D., N. M. Taylor, and M. A. Kossaibati. 2013. Is targeted milk sampling an effective means of detecting Johne's disease in dairy herds? Cattle Pract. 22:26–34.

HerdWise. 2022. HerdWise rule change. Accessed Feb. 2, 2020. https://www.nmr.co.uk/uploads/files/files/HW%20Classification%20change%20farmer(1).pdf.

Kennedy, A. E., A. T. Da Silva, N. Byrne, R. Govender, J. MacSharry, J. O'Mahony, and R. G. Sayers. 2014. The single intradermal cervical comparative test interferes with Johne's disease ELISA diagnostics. Front. Immunol. 5:564. https://doi.org/10.3389/fimmu.2014.00564.

Laurin, E. L., J. Sanchez, M. Chaffer, S. McKenna, and G. Keefe. 2017. Assessment of the relative sensitivity of milk ELISA for detection of *Mycobacterium avium* ssp. *paratuberculosis* infectious dairy cows. J. Dairy Sci. 100:598–607. https://doi.org/10.3168/jds.2016-11194.

Li, L., J. Bannantine, J. Campo, A. Randall, Y. Grohn, R. Katani, M. Schilling, J. Radzio-Basu, and V. Kapur. 2017. Identification of sero-reactive antigens for the early diagnosis of Johne's disease in cattle. PloS One 12:0184373. https://doi.org/10.1371/journal.pone.0184373.

Lilenbaum, W., R. Ferreira, C. Marassi, P. Ristow, W. Oelemann, and L. Fonseca. 2007. Interference of tuberculosis on the performance of ELISAs used in the diagnosis of paratuberculosis in cattle. Braz. J. Microbiol. 38:472–477. https://doi.org/10.1590/S1517-83822007000300016.

Lombard, J. E. 2011. Epidemiology and economics of paratuberculosis. Vet. Clin. North Am. Food Anim. Pract. 27:525–535. https://doi.org/10.1016/j.cvfa.2011.07.012.

May, H., P. Orpin, H. Pearse, P. Jones, and H. Clough. 2016. The influence of tuberculosis testing in dairy cattle on milk ELISA tests for Johne's disease. Accessed Nov. 11, 2019. https://www.researchgate.net/profile/Peter_Orpin2/publication/237445560_The_influence_of_tuberculosis_testing_in_dairy_cattle_on_milk_ELISA_tests_for_Johne's_disease/links/56dddae908aeb8b66f94a03f/The-influence-of-tuberculosis-testing-in-dairy-cattle-on-milk-ELISA-tests-for-Johnes-disease.pdf.

Meyer, A., K. Bond, S. Van Winden, M. Green, and J. Guitian. 2018. A probabilistic approach to the interpretation of milk antibody results for diagnosis of Johne's disease in dairy cattle. Prev. Vet. Med. 150:30–37. https://doi.org/10.1016/j.prevetmed.2017.11.016.

Monaghan, M. L., M. Doherty, J. Collins, J. Kazda, and P. Quinn. 1994. The tuberculin test. Vet. Microbiol. 40:111–124. https://doi.org/10.1016/0378-1135(94)90050-7.

Nielsen, S. S. 2008. Transitions in diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* infections in cattle. Vet. Microbiol. 132:274–282. https://doi.org/10.1016/j.vetmic.2008.05.018.

Nielsen, S. S., C. Enevoldsen, and Y. T. Gröhn. 2002a. The *Mycobacterium avium* subsp. *paratuberculosis* ELISA response by parity and stage of lactation. Prev. Vet. Med. 54:1–10. https://doi.org/10.1016/S0167-5877(02)00008-9.

Nielsen, S. S., C. Grønbæk, J. Agger, and H. Houe. 2002b. Maximum-likelihood estimation of sensitivity and specificity of ELISAs and faecal culture for diagnosis of paratuberculosis. Prev. Vet. Med. 53:191–204. https://doi.org/10.1016/S0167-5877(01)00280-X.

Nielsen, S. S., and N. Toft. 2009. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev. Vet. Med. 88:1–14. https://doi.org/10.1016/j.prevetmed.2008.07.003.

Ott, S. L., S. J. Wells, and B. A. Wagner. 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet. Med. 40:179–192. https://doi.org/10.1016/S0167-5877(99)00037-9.

Palmer, M. V., W. Waters, T. Thacker, R. Greenwald, J. Esfandiari, and K. Lyashchenko. 2006. Effects of different tuberculin skin-testing regimens on gamma interferon and antibody responses in cattle experimentally infected with *Mycobacterium bovis*. Clin. Vaccine Immunol. 13:387–394. https://doi.org/10.1128/CVI.13.3.387-394.2006.

Patterson, S., K. Bond, M. Green, S. van Winden, and J. Guitian. 2020. *Mycobacterium avium paratuberculosis* infection of calves – The impact of dam infection status. Prev. Vet. Med. 181:104634. https://doi.org/10.1016/j.prevetmed.2019.02.009.

Picasso-Risso, C., A. Grau, D. Bakker, J. Nacar, O. Mínguez, A. Perez, and J. Alvarez. 2019. Association between results of diagnostic tests for bovine tuberculosis and Johne's disease in cattle. Vet. Rec. 185:693. https://doi.org/10.1136/vr.105336.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org/.

Radia, D., K. Bond, G. Limon, S. van Winden, and J. Guitian. 2013. Relationship between periparturient management, prevalence of MAP and preventable economic losses in UK dairy herds. Vet. Rec. 173:343. https://doi.org/10.1136/vr.101408.

Rathnaiah, G., D. Zinniel, J. Bannantine, J. Stabel, Y. Gröhn, M. Collins, and R. Barletta. 2017. Pathogenesis, molecular genetics, and genomics of *Mycobacterium avium* subsp. *paratuberculosis*, the etiologic agent of Johne's Disease. Front. Vet. Sci. 4:187. https://doi.org/10.3389/fvets.2017.00187.

Roupie, V., E. Alonso-Velasco, S. Van Der Heyden, S. Holbert, L. Duytschaever, P. Berthon, I. Van Dosselaer, W. Van Campe, L. Mostin, F. Biet, S. Roels, K. Huygen, and D. Fretin. 2018. Evaluation of mycobacteria-specific gamma interferon and antibody responses before and after a single intradermal skin test in cattle naturally exposed to *M. avium* subsp. *paratuberculosis* and experimentally infected with *M. bovis*. Vet. Immunol. Immunopathol. 196:35–47. https://doi.org/10.1016/j.vetimm.2017.12.007.

Sechi, L. A., and C. Dow. 2015. *Mycobacterium avium* ss. *paratuberculosis* zoonosis – The hundred year war – Beyond Crohn's disease. Front. Immunol. 6:96. https://doi.org/10.3389/fimmu.2015.00096.

Seva, J., J. Sanes, G. Ramis, A. Mas, J. Quereda, B. Villarreal-Ramos, D. Villar, and F. Pallares. 2014. Evaluation of the single cervical skin test and interferon gamma responses to detect Mycobacterium avium subsp. *paratuberculosis* infected cattle in a herd co-infected with *Mycobacterium avium* subsp. *paratuberculosis*. Vet. Microbiol. 171:139–146. https://doi.org/10.1016/j.vetmic.2014.03.035.

Sibley, R. 2019. The future of controlling and eradicating TB from infected cattle herds. Livestock (Lond.) 24:137–141. https://doi.org/10.12968/live.2019.24.3.137.

Stabel, J. R., S. Wells, and B. Wagner. 2002. Relationships between fecal culture, ELISA, and bulk tank milk test results for Johne's disease in US dairy herds. J. Dairy Sci. 85:525–531. https://doi.org/10.3168/jds.S0022-0302(02)74104-0.

Stabel, J. R., K. Kimura, and S. Robbe-Austerman. 2007. Augmentation of secreted and intracellular gamma interferon following johnin

purified protein derivative sensitization of cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*. J. Vet. Diagn. Invest. 19:43–51. https://doi.org/10.1177/104063870701900107.

Sweeney, R. W. 1996. Transmission of paratuberculosis. Vet. Clin. North Am. Food Anim. Pract. 12:305–312. https://doi.org/10.1016/S0749-0720(15)30408-4.

Sweeney, R. W. 2011. Pathogenesis of paratuberculosis. Vet. Clin. North Am. Food Anim. Pract. 27:537–546. https://doi.org/10.1016/j.cvfa.2011.07.001.

Tameni, S., M. Amadori, P. Scaccaglia, R. Quondam-Giandomenico, S. Tagliabue, I. L. Achetti, R. Adone, and F. Ciuchini. 1998. Quality controls and in vitro diagnostic efficiency of bovine PPD tuberculins. Biologicals 26:225–235. https://doi.org/10.1006/biol.1998.0147.

TB hub. 2021. The anamnestic boosting effect of the skin test on antibody responses to *Mycobacterium bovis* in camelids – Summary of the evidence. Accessed Mar. 10, 2022. https://tbhub.co.uk/wp-content/uploads/2021/11/Anamnestic_antibody_response_scientific_evidence.pdf.

Therneau, T. 2020. coxme: Mixed Effects Cox Models. R package version 2.2–16. https://CRAN.R-project.org/package=coxme.

Toft, N., S. Nielsen, and E. Jørgensen. 2005. Continuous-data diagnostic tests for paratuberculosis as a multistage disease. J. Dairy Sci. 88:3923–3931. https://doi.org/10.3168/jds.S0022-0302(05)73078-2.

Varges, R., C. Marassi, W. Oelemann, and W. Lilenbaum. 2009. Interference of intradermal tuberculin tests on the serodiagnosis of paratuberculosis in cattle. Res. Vet. Sci. 86:371–372. https://doi.org/10.1016/j.rvsc.2008.08.006.

Velasova, M., A. Damaso, B. Prakashbabu, J. Gibbons, N. Wheelhouse, D. Longbottom, S. Van Winden, M. Green, and J. Guitian. 2017. Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain. J. Dairy Sci. 100:9215–9233. https://doi.org/10.3168/jds.2016-11863.

Whittington, R., K. Donat, M. F. Weber, D. Kelton, S. S. Nielsen, S. Eisenberg, N. Arrigoni, R. Juste, J. L. Sáez, N. Dhand, A. Santi, A. Michel, H. Barkema, P. Kralik, P. Kostoulas, L. Citer, F. Griffin, R. Barwell, M. A. S. Moreira, I. Slana, H. Koehler, S. V. Singh, H. S. Yoo, G. Chávez-Gris, A. Goodridge, M. Ocepek, J. Garrido, K. Stevenson, M. Collins, B. Alonso, K. Cirone, F. Paolicchi, L. Gavey, M. T. Rahman, E. de Marchin, W. Van Praet, C. Bauman, G. Fecteau, S. McKenna, M. Salgado, J. Fernández-Silva, R. Dziedzinska, G. Echeverría, J. Seppänen, V. Thibault, V. Fridriksdottir, A. Derakhshandeh, M. Haghkhah, L. Ruocco, S. Kawaji, E. Momotani, C. Heuer, S. Norton, S. Cadmus, A. Agdestein, A. Kampen, J. Szteyn, J. Frössling, E. Schwan, G. Caldow, S. Strain, M. Carter, S. Wells, M. Munyeme, R. Wolf, R. Gurung, C. Verdugo, C. Fourichon, T. Yamamoto, S. Thapaliya, E. Di Labio, M. Ekgatat, A. Gil, A. N. Alesandre, J. Piaggio, A. Suanes, and J. H. de Waard. 2019. Control of paratuberculosis: Who, why and how. A review of 48 countries. BMC Vet. Res. 15:198. https://doi.org/10.1186/s12917-019-1943-4.

Whittington, R. J., and P. Windsor. 2009. In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis. Vet. J. 179:60–69. https://doi.org/10.1016/j.tvjl.2007.08.023.

Windsor, P., and R. J. Whittington. 2010. Evidence for age susceptibility of cattle to Johne's disease. Vet. J. 184:37–44. https://doi.org/10.1016/j.tvjl.2009.01.007.

Wood, S. N. 2017. Generalized Additive Models: An Introduction with R. 2nd ed. Chapman and Hall/CRC.

ORCIDS

E. Nunney ⓘ https://orcid.org/0000-0001-6574-0147

M. Crotta ⓘ https://orcid.org/0000-0002-5508-9028

S. van Winden ⓘ https://orcid.org/0000-0002-7321-4996

K. Bond ⓘ https://orcid.org/0000-0002-9050-6923

M. Green ⓘ https://orcid.org/0000-0002-6408-6443

J. Guitian ⓘ https://orcid.org/0000-0003-0799-0476