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Associations between Johne's disease and fertility in UK dairy herds

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ABSTRACT

The objective of this observational study was to quantify associations between *Mycobacterium avium* subspecies *paratuberculosis* (MAP) antibody status and a variety of fertility outcomes, in UK dairy cattle. Longitudinal milk recording, fertility and MAP antibody enzyme-linked immunosorbent assay (ELISA) milk test data were collated retrospectively from 121,762 lactations in 78 herds. Datasets were structured into appropriate units to suit outcomes and enable temporal association between current and future MAP status, and fertility measures. Current MAP status was categorised according to most recent status within 180 days, with time-related future MAP status assigned based on MAP antibody ELISA milk test data for each cow. Multilevel multivariable logistic regression models were used to evaluate associations between MAP status and 21-day pregnancy and submission rate and conception risk. Posterior predictions and cross-validation techniques were used to assess model fit and check model building assumptions. A negative association was found between risk of insemination (Odds Ratio [OR], 0.78; 95% Credible Interval [CI], 0.66–0.92) and conception occurring (OR, 0.65; CI, 0.5–0.84) and transition from negative to non-negative MAP test status in the next 30–90 days. A positive association was observed between risk of insemination (OR, 1.34; CI, 1.16–1.52) and conception occurring (OR, 1.26; CI, 1.11–1.43) and transition from negative to non-negative MAP test status in the next 90–180 days. Current positive MAP test status was negatively and positively associated with insemination (OR, 0.59; CI, 0.49–0.70) and conception risk (OR, 1.12; CI, 0.96–1.30), respectively. Herd managers will have had access to test results, declaring cows with past recent or multiple positive MAP antibody ELISA results not to be bred, negatively influencing insemination risk. Overall, these results demonstrate the temporal association between a positive MAP antibody ELISA result and dairy cow fertility outcomes, with particular variability prior to a positive MAP antibody ELISA result.

Introduction

Paratuberculosis (Johne's disease) is a fatal, transmissible and chronic condition of both ruminants and non-ruminants, characterised by inflammatory granulomatous enteritis and caused by Gram-positive *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Sweeney, 2011).

Paratuberculosis has both significant positive and negative associations with cow health and productivity; with particular consistent negative impacts reported on milk production and more recently, on udder health (McAloon et al., 2016; Pritchard et al., 2017; Rossi et al., 2017; Martins et al., 2018). Variable associations with fertility have been reported (McAloon et al., 2019), as have an increased incidence of lameness associated with paratuberculosis (Raizman et al., 2007; Villarino and Jordan, 2005), premature culling (Raizman et al., 2009; Smith et al., 2010) and reduced slaughter value (Richardson and More, 2009). Clinical cases are typified by a two-year plus incubation, progressing to weight loss, diarrhoea, dehydration and cachexia (Fecteau, 2018; Sweeney, 2011). Substantial cow and farmer welfare implications associated with clinical cases must be considered (McAloon et al., 2017).

Diagnosis of MAP proves challenging with limited sensitivity of currently available tests (Nielsen and Toft, 2008); both serum antibody enzyme-linked immunosorbent assay (ELISA) and milk antibody ELISA are utilised for serial prevalence monitoring in dairy herds (Garcia and Shalloo, 2015), with faecal PCR utilised as a confirmatory test (Clark et al., 2008) and faecal culture still recognised as a reference test (Barrett et al., 2011).

Suboptimal fertility can contribute significant cost to dairy herds (Cabrera, 2014), however associations between reproductive outcomes and subclinical paratuberculosis are unclear. Prospective, limited scale

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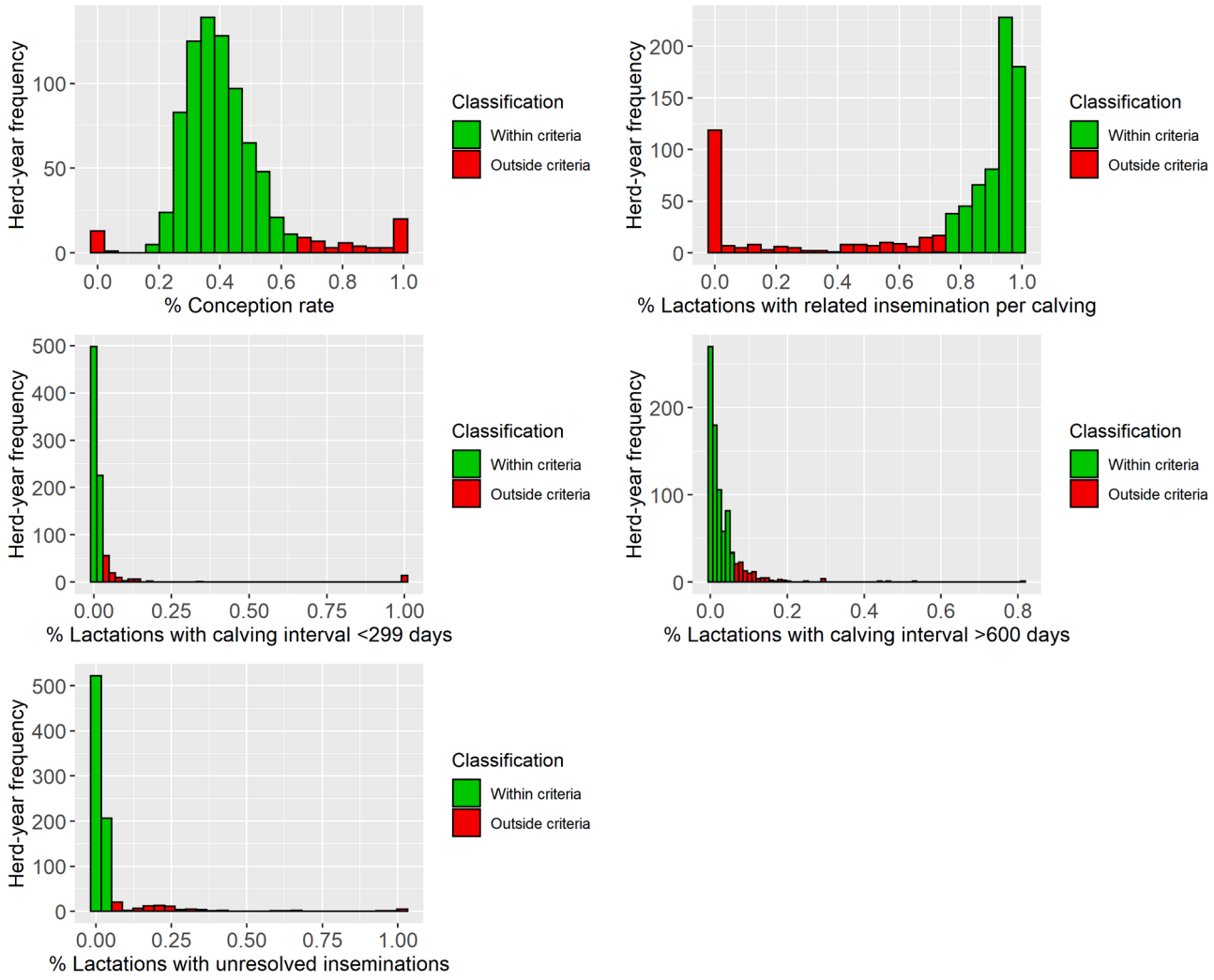

Fig. 1. Distribution of data quality measures across herd-years; the proportion of lactations with an unrelated insemination per calving and a physiologically plausible calving interval and the proportion of inseminations with unresolved and successful outcomes.

studies with controlled, detailed recording of events demonstrate mainly negative association between paratuberculosis and dairy cow fertility (Johnson-Ifearulundu et al., 2000; Raizman et al., 2007); however larger scale epidemiological studies with cruder or more potentially biased outcome measures demonstrate either positive (Lombard et al., 2005; Gonda et al., 2007; Marcé et al., 2009; Smith et al., 2010) or unclear (Pritchard et al., 2017) association between paratuberculosis and fertility outcomes. Current knowledge is also limited by use of interval-based measures of fertility performance, presenting challenges for analysis of a progressive disease where infected animals can be culled before the end of a lactation. Furthermore, an inability to link test positivity temporally to fertility indices in most studies, hinders exploration of the association between MAP test status and fertility.

Given the equivocal existing evidence on association between fertility and MAP test status and potential significant economic implications, further research into the effect of subclinical paratuberculosis on reproductive efficiency is warranted. The aim of this study was to evaluate the association between milk antibody ELISA MAP test status and overall fertility performance, across a large sample of commercial dairy herds from the United Kingdom. Risk of insemination and conception occurring temporally relative to MAP test status was assessed using two different models to determine the contribution of each of these factors to associations between MAP test status and overall fertility performance. A third model represented a culmination of associations between MAP test status and insemination and conception risk.

Materials and methods

Data collection and restructuring

The study protocol was approved by the University of Nottingham School of Veterinary Medicine and Science Ethical Review Committee (Approval number, 2838 190927; Approval date, 28 August 2019).

Longitudinal milk recording, fertility and MAP antibody ELISA milk test data were collated from 93 UK dairy herds from the database of a national milk recording organisation (Quality Milk Management Services Ltd). No data regarding herd management practices were available. Data were anonymised and converted into a consistent database format. The initial dataset consisted of data from 325,707 lactations in 95,823 cows, beginning in the years 1978–2020.

Novel data quality assessment criteria were developed and applied to each data set. Each dataset was initially screened for duplicate event records (calving, insemination, pregnancy diagnosis, dry off and *Mycobacterium avium* subspecies *paratuberculosis* [MAP] antibody enzyme-linked immunosorbent assay [ELISA] test data). Further assessment criteria were applied at herd-year level, facilitating removal of herds with missing event data or physiologically implausible data, considered to have potential to impact results (Fig. 1). These included measurement of the proportion of lactations with an unrelated insemination per calving and a physiologically plausible calving interval, alongside measurement of proportion of inseminations with unresolved outcomes.

Table 1
Summary of production and fertility metrics and Mycobacterium subspecies avium paratuberculosis (MAP) antibody milk enzyme-linked immunosorbent assay (ELISA) testing characteristics for 78 herds used in model building[a].

Parameter	Mean	Min	Max	25% quantile	50% quantile	75% quantile
Herd size	299	53	1292	141	229	363
Predicted 305-day milk yield (kg)	8563	4421	11,832	7373	8707	9731
Culling rate (%/year)	23.75	0	44.02	19.37	24.56	29.46
Calving interval (days)	398	365	433	391	398	408
Conception rate (%)	39.77	26.33	63.21	33.63	38.57	44.62
21-day pregnancy rate (%)	13.74	8.46	21.71	11.81	13.50	15.01
21-day submission rate (%)	33.93	21.66	57.15	28.81	33.93	37.40
Days to first insemination (days)	74	56	97	69	73	79
Days to conception (days)	118	87	154	110	119	126
MAP antibody milk ELISA tests per lactation	1.72	0	5.74	0.80	1.82	2.59
Lactations with > 1 MAP antibody milk ELISA test (%)	53.88	0	100	8.54	53.88	86.86
Lactations with > 2 MAP antibody milk ELISA tests (%)	31.42	0	95.58	0.61	22.71	59.25
Days between MAP antibody milk ELISA	132	33	609	97	116	146

MAP, Mycobacterium subspecies avium paratuberculosis; ELISA, enzyme-linked immunosorbent assay.
[a]The mean value was calculated for each herd over the time period that herd featured in the data set; the values in the table describe the distribution of these herd values.

Apparent conception rate was analysed to detect systematic under-recording of herd insemination data. Lactation level criteria were also applied and lactations were only retained with a calving event that occurred between 01/01/2010 and 30/09/2018. Basic statistics describing the datasets before and after data quality assessment are included in Supplementary Tables S1 and S2. Following screening of data, 121,762 lactations including 266,246 inseminations in 51,345 cows from 78 herds were retained for further analysis. Basic statistics describing these herds in more detail are provided in Tables 1 and 2.

Fertility and MAP milk antibody ELISA test data were amalgamated and two datasets constructed. Restructuring was carried out in R version 4.0.3 (R Core Team, 2020) using the tidyverse package (Wickham et al., 2019). Dataset 1 was restructured into a format where each unit of data represented a 21-day risk period in every lactation between 21 and 336 days-in-milk (DIM) to evaluate the association between MAP test status and risk of insemination and pregnancy occurring per risk period. Dataset 2 was restructured such that insemination events were the units of data to evaluate association between MAP test status and risk of conception occurring for a given insemination. Occurrence of insemination and pregnancy in each 21-day risk period and occurrence of pregnancy for each insemination were included as binary variables based upon recorded events; if no pregnancy diagnosis was recorded then pregnancy was determined to have occurred where a calving was recorded 257–307 days after an insemination (Hudson et al., 2012). In the case of multiple successive inseminations fulfilling this criteria (7.96% lactations), the closest insemination to average dairy gestation length was considered the successful one (Norman et al., 2009). Risk periods were censored from analysis when cows were no longer eligible for insemination (i.e. if pregnant or marked not to breed). For each risk period and insemination, multiple potential explanatory variables were also calculated, listed in Table 3.

Milk samples were tested for MAP antibody via the commercial MAP IDEXX milk antibody ELISA (*Mycobacterium paratuberculosis* Antibody Test Kit, IDEXX Europe (Buddle et al., 2013; Fry et al., 2008)). Classification of MAP milk antibody ELISA test results and categorisation into risk-based status based upon testing history are defined in Table 4. The closest MAP test status was allocated to each risk period or insemination, if there was no MAP test status within 180 days of the end of the risk period or insemination, the MAP test status for this was coded as missing. Additional binary variables were constructed for each risk period and insemination, representing the cow moving from a negative to a non-negative MAP test status within various timeframes following the risk period or insemination.

Statistical analysis

Model 1

A logistic regression model with the binary event of pregnancy becoming established as the outcome variable was used to evaluate the association between the probability of a cow becoming pregnant in a given 21-day risk period, MAP test status and other potential explanatory variables. A multilevel hierarchical model was used with a three-level structure to account for correlations within the data, with risk periods nested within cows nested within herds. Data from dataset one were used to construct the model (see Supplementary Data: Materials and methods, for model specification).

Model building was carried out using the lme4 package (Bates et al., 2015) in R, using iterative generalised least squares for initial parameter estimation. Initial model building was via stepwise forward selection, with non-MAP test status explanatory variables (i.e. lactation number, DIM, 305-day yield) added before MAP test status variables. Explanatory variables were retained in the model if the 95% confidence interval for

Table 2
Summary of number of *Mycobacterium* subspecies *avium paratuberculosis* (MAP) antibody milk enzyme-linked immunosorbent assay (ELISA) tests in data set[a].

	MAP test status	Lactation 1	Lactation 2	Lactation 3	Lactation 4	>Lactation 4
Number of MAP milk ELISA tests	Currently negative	60,285	47,599	31,594	19,759	23,938
	Uncertain	1578	2086	1916	1347	1858
	Provisionally Positive	873	1081	795	573	686
	Positive	203	581	611	584	791
Number of cows	Transition to Non-Negative from Currently Negative MAP test status[b]	293	423	340	214	235

MAP, Mycobacterium subspecies avium paratuberculosis; ELISA, enzyme-linked immunosorbent assay
[a]Grouped by MAP test status and parity, and number of cows transitioning from Currently Negative to Non-Negative MAP test status, across different parities
[b]Uncertain/Provisionally Positive/Positive MAP test status

Table 3
Variables and variable type calculated at each level of data per 21-day risk period or insemination in datasets 1 and 2 respectively.

Variable	Variable type
21-day risk period or insemination level	
Inseminated[a]	Binary (served or not served)
Insemination outcome	Binary (becomes pregnant or does not)
DIM at start of risk period/at insemination	Continuous
Month of insemination/risk period	Categorical; January, February, March, April, May, June, July, August, September, October, November, December
Year of insemination/risk period	Categorical; 2010–2019
ISI[b]	Continuous
ISI category[b]	Categorical; < 19 days, 19–26 days, 27–37 days, 38–52 days, > 52 days, none ~ (first insemination, missing)
Insemination number[b]	Categorical; 1,2,3,4 or > 4
MAP test status	Categorical; Currently negative, Uncertain, Provisionally Positive, Positive, No status – (missing)
Currently Negative to non-negative MAP test status within next 30 days	Binary indicator representing increase from Currently Negative to non-negative MAP test status within 30 days after end of risk period/insemination or not
Currently Negative to non-negative MAP test status within next 30–90 days	Binary indicator representing increase from Currently Negative to non-negative MAP test status within 30–90 days after end of risk period/insemination or not
Currently Negative to non-negative MAP test status within next 90–180 days	Binary indicator representing increase from Currently Negative to non-negative MAP test status within 90–180 days after end of risk period/insemination or not
Currently Negative to non-negative MAP test status within next 180–365 days	Binary indicator representing increase from Currently Negative to non-negative MAP test status within 30 days after end of risk period/insemination or not
Currently Negative to non-negative MAP test status after more than 365 days	Binary indicator representing increase from Currently Negative to non-negative MAP test status within 365 days after end of risk period/insemination or not
Lactation level	
Month in which lactation began	Categorical; January, February, March, April, May, June, July, August, September, October, November, December
Year in which lactation began	Categorical; 2010–2018
Parity of cow	Categorical (1,2,3,4, or >4)
Previous lactation MAP test status	Categorical; Currently negative, Uncertain, Provisionally Positive, Positive, No status- (missing)
305-day milk yield (x 1000 kg)	Continuous

ISI, Interservice Interval; MAP, Mycobacterium subspecies avium paratuberculosis
[a]Applicable to 21-day risk period level only (dataset 1).
[b]Applicable to insemination level only (dataset 2).

their coefficients did not cover zero and if rejected, were reintroduced into the model after initial variable selection and retained if they satisfied the criteria described above. Where one or more categories of a categorical variable were significant, all categories for that variable were retained. First order interactions between explanatory variables were only tested if considered clinically important and polynomial functions tested for all continuous variables up to power three. Interactions and polynomial functions were retained where the 95% confidence interval for their coefficients did not cover zero. Shapes described by polynomial functions were also compared to patterns in raw data to ensure appropriate shapes were fitted. Final parameter estimates were generated with Markov chain Monte Carlo (MCMC) using the brms package (Bürkner, 2021, 2018, 2017) using two chains and 2000 iterations per chain after a 1000 iteration burn in. Initial parameter values for chains were randomly generated from within appropriate parameter space. The Gelman-Rubin statistic was used to evaluate chain convergence, with values close to one indicating satisfactory convergence. MCMC was used for final parameter estimation due to increased likely reliability of estimates (Browne and Draper, 2006) alongside provision of model fit assessment via generation of full posterior predictions.

Table 4
Definition of *Mycobacterium* subspecies *avium paratuberculosis* (MAP) test status.

MAP test status	Definition
Currently negative	All tests negative (antibody titre less than sample-to-positive control ratio of 0.2) or previously tested Uncertain/ Provisionally Positive once but have subsequently tested negative on three consecutive occasions
Uncertain	Antibody titre is equal to or more than the sample-to-positive control ratio of 0.2 and less than 0.3 and/or cows that have previously tested Positive (antibody titre equal to or more than sample-to-positive control ratio of 0.3) but have since tested negative on less than three consecutive occasions
Provisionally Positive	The latest MAP ELISA milk test is Positive
Positive	Two MAP ELISA milk test Positives out of the last three tests; will not be re-classified once categorised as Positive, regardless of subsequent MAP ELISA results

MAP, Mycobacterium subspecies avium paratuberculosis; ELISA, enzyme-linked immunosorbent assay

Model 2

To evaluate the association between the probability of a cow being inseminated in a given 21-day risk period, MAP test status and other potential explanatory variables, another logistic regression model was constructed with the binary event of occurrence of insemination as the outcome variable. The same dataset, model specification and building process was used to construct this model as those used for Model 1.

Model 3

To assess the association between the probability of a cow becoming pregnant as a result of a given insemination, MAP test status and other potential explanatory variables, a third logistic regression model was constructed with the binary event of insemination success as the outcome variable. Data from dataset two were used to construct this model (see Supplementary Data: Materials and methods, for model specification). Model building was carried out as previously described.

Model assumption checking and assessment of model fit

Posterior predictive assessment was used to evaluate model fit (Green et al., 2009) and cross-validation used to check for model overfitting; full posterior predictions were used to calculate illustrative predicted relative risks for each model (see Supplementary Data: Materials and methods).

Results

Model 1

Assessing the total of 365,142 21-day risk periods under analysis after excluding risk periods with missing 305-day yield data, mean probability of pregnancy occurring in a given risk period was 0.140 (see Supplementary Table S3).

Parameter and variance estimates for Model 1 are shown in Table 5; estimates for odds ratios were calculated by exponentiation of coefficient estimates. Parity, 305-day yield and month of 21-day risk period and calving were explanatory variables included in final analysis; all were significantly associated with the probability of a cow becoming pregnant during a risk period.

All current and previous lactation non-negative MAP antibody ELISA status were negatively associated with probability of pregnancy

Table 5
Parameter estimates for logistic regression Models 1 [a],2 [b] and 3 [c].

	Model 1		Model 2		Model 3	
Characteristic	OR	95% CI	OR	95% CI	OR	95% CI
305-day milk yield (1000 kg)	1.11	1.07, 1.14	1.21	1.17, 1.23	0.91	0.90, 0.92
(305-day milk yield (1000 kg)) squared	0.99	0.99, 0.99	0.99	0.99, 0.99		
Lactation number						
1	Reference		Reference		Reference	
2	0.87	0.83, 0.90	0.90	0.86, 0.93	0.90	0.86, 0.92
3	0.76	0.72, 0.79	0.74	0.71, 0.76	0.84	0.81, 0.88
4	0.60	0.57, 0.63	0.57	0.54, 0.59	0.76	0.73, 0.80
> 4	0.42	0.40, 0.44	0.39	0.37, 0.41	0.64	0.62, 0.68
Current MAP test status						
Currently Negative	Reference		Reference		Reference	
Uncertain	0.94	0.87, 1.02	0.98	0.91, 1.04	1.03	0.96, 1.11
Provisionally Positive	0.77	0.70, 0.84	0.70	0.65, 0.76	0.95	0.85, 1.06
Positive	0.64	0.52, 0.79	0.59	0.49, 0.70	1.12	0.96, 1.30
No status	0.97	0.94, 0.99	0.90	0.90, 0.93	0.92	0.89, 0.97
Previous lactation MAP test status						
Currently Negative	Reference		Reference			
Uncertain	1.00	0.92, 1.08	0.95	0.89, 1.01		
Provisionally Positive	0.86	0.76, 0.95	0.78	0.70, 0.86		
Positive	0.66	0.53, 0.81	0.52	0.43, 0.62		
No status	1.00	0.96, 1.04	0.99	0.96, 1.02		
Currently Negative to non-negative[d] MAP test status within 30–90 days after end of risk period (Model 1 and 2) or insemination (Model 3)	0.64	0.53, 0.79	0.78	0.66, 0.92	0.65	0.50, 0.84
Currently Negative to non-negative MAP test status within 90–180 days after end of risk period (Model 1 and 2) or insemination (Model 3)	1.46	1.26, 1.68	1.34	1.16, 1.52	1.26	1.11, 1.43
Currently Negative to non-negative MAP test status within next 180–365 days after end of risk period (Model 1 and 2) or insemination (Model 3)	1.72	1.36, 2.12	1.70	1.40, 2.08	1.08	0.96, 1.22
Currently Negative to non-negative MAP test status in > 365 days after end of risk period (Model 1 and 2) or insemination (Model 3)	2.14	1.27, 3.53				
DIM						
21–41	Reference		Reference			
42–62	8.94	8.14, 9.49	11.02	11.02, 11.02		
63–83	14.44	13.60, 15.49	18.17	18.17, 20.09		
84–104	18.17	16.95, 19.30	24.53	22.20, 24.53		
105–125	16.95	15.80, 18.17	20.09	20.09, 22.20		
126–146	14.87	13.87, 15.96	18.17	16.44, 18.17		
147–167	13.74	12.81, 14.88	14.88	14.88, 16.44		
168–188	11.82	11.02, 12.81	12.18	12.18, 13.46		
189–209	11.25	2.34, 2.51	11.02	11.02, 12.18		
210–230	9.68	10.38,10.59	9.97	9.02, 9.97		
231–251	9.12	8.25, 9.97	8.17	7.39, 9.03		
252–272	8.00	7.17, 8.85	6.69	6.05, 6.69		
273–293	5.64	5.00, 6.36	4.95	4.48, 5.47		
294–314	4.57	4.01, 5.26	3.32	3.00, 3.67		
315–335	3.35	2.89, 3.94	2.46	2.23, 2.72		
ln DIM					6.69	4.48, 11.02
(ln DIM) squared					0.83	0.79, 0.87
ISI						
None					Reference	
0–19 days					0.72	0.68, 0.76
19–26 days					1.21	1.17, 1.26
27–37 days					0.97	0.91, 1.03
38–52 days					0.99	0.94, 1.04
> 53 days					1.09	1.03, 1.15
Risk period months 2–10	0.74	0.73, 0.76				
Risk period month 1			1.38	1.34, 1.42		
Risk period month 5			1.54	1.49, 1.58		
Risk period month 11 and 12			1.60	1.57, 1.63		
Calving year 2017					0.92	0.90, 0.95
Calving months 3,6, and 7	1.02	1.00, 1.05				
Insemination year 2017 and 2018					1.06	1.03, 1.09
Insemination months 3,7,8,9 and 10					0.95	0.92, 0.97

OR, Odds Ratio; CI, Credible Interval; MAP, Mycobacterium subspecies avium paratuberculosis; DIM, Days-In-Milk; ISI, Interservice Interval

[a] Model outcome; cow becoming pregnant in 21-day risk period. Herd level variance was estimated as 0.26 (0.025 standard error) and cow level variance estimated as 0.39 (0.016 standard error).

[b] Model outcome; cow receiving insemination in 21-day risk period. Herd level variance was estimated as 0.38 (0.035 standard error) and cow level variance estimated as 0.65 (0.007 standard error).

[c] Model outcome; cow becoming pregnant for given insemination. Herd level

variance was estimated as 0.31 (0.029 standard error) and cow level variance estimated as 0.48 (0.016 standard error).
[d] Uncertain/Provisionally Positive/Positive MAP test status.

occurring in a given 21-day risk period compared to Currently Negative status, except previous lactation Uncertain status. Magnitude of negative association was largest and significant in MAP test status defined by the highest number of recent Positive MAP antibody ELISA results (largest reduction in odds of cow becoming pregnant in a risk period associated with Positive status; Odds Ratio [OR], 0.64; 95% Credible Interval [CI], 0.52–0.79); this pattern was mirrored with Provisionally Positive and Positive previous lactation MAP antibody ELISA status significantly negatively associated with probability of pregnancy occurring in a given risk period. Transition from Currently Negative MAP antibody ELISA status to any non-negative MAP antibody ELISA status in the next 30–90 days after the end of a risk period was significantly negatively associated with probability of pregnancy occurring (OR, 0.64; CI, 0.53–0.79); transition from Negative to Positive MAP test status in the next 90–180 days (OR, 1.46; CI, 1.26–1.68), 180–365 days (OR, 1.72; CI, 1.36–2.12) and more than 365 days after the end of a risk period (OR, 2.14; CI, 1.27–3.53) was significantly positively associated with probability of pregnancy occurring.

Model 2

Assessing the 21-day risk periods as described in Model 1, mean probability of insemination occurring in a given risk period was 0.364 (see Supplementary Table S3).

The same explanatory variables were included in Model 2 as in Model 1, expect for the addition of year of risk period. Parameter and variance estimates for Model 2 are shown in Table 5.

Associations between current and previous lactation Provisionally Positive and Positive MAP antibody ELISA status and probability of insemination occurring in a given risk period were again significantly negative but of a greater magnitude than those described in Model 1. Associations between transition from Currently Negative to any non-negative MAP antibody ELISA status and probability of insemination occurring were also of a similar direction to those described in Model 1, with Positive MAP test status transition in the next 30–90 days after the end of a risk period demonstrating a smaller negative association with probability of insemination occurring and Positive MAP test status transition in the next 90–180 days demonstrating a small positive association with probability of insemination occurring, compared to Model 1. In contrast to Model 1, in Model 2 transition to a non-negative status at > 365 days after the end of a risk period had no significant association with probability of insemination occurring.

Model 3

A total of 136,691 inseminations were assessed in Model 3 after excluding inseminations with missing 305-day yield data, with an overall conception risk (risk of an insemination leading to a pregnancy) of 37.67%. Parameter and variance estimates for Model 3 are shown in Table 5.

None of current MAP antibody ELISA status categories were significantly different from the reference category (Currently Negative MAP antibody ELISA status), with only the No Status category significantly different from the reference. Similarly to Model 1, transition from Currently Negative MAP antibody ELISA status to any non-negative MAP antibody ELISA status in the next 30–90 days after an insemination was significantly negatively associated with probability of pregnancy occurring (OR, 0.65; CI, 0.50–0.84) and transition from Currently Negative to any non-negative MAP test status in the next 90–180 days after an insemination (OR, 1.26; CI, 1.11–1.43) was significantly positively associated with probability of pregnancy occurring. Transition from Currently Negative to any non-negative MAP test status in the next 180–365 days after an insemination (OR, 1.08; CI, 0.96–1.22) demonstrated a positive association with probability of pregnancy occurring,

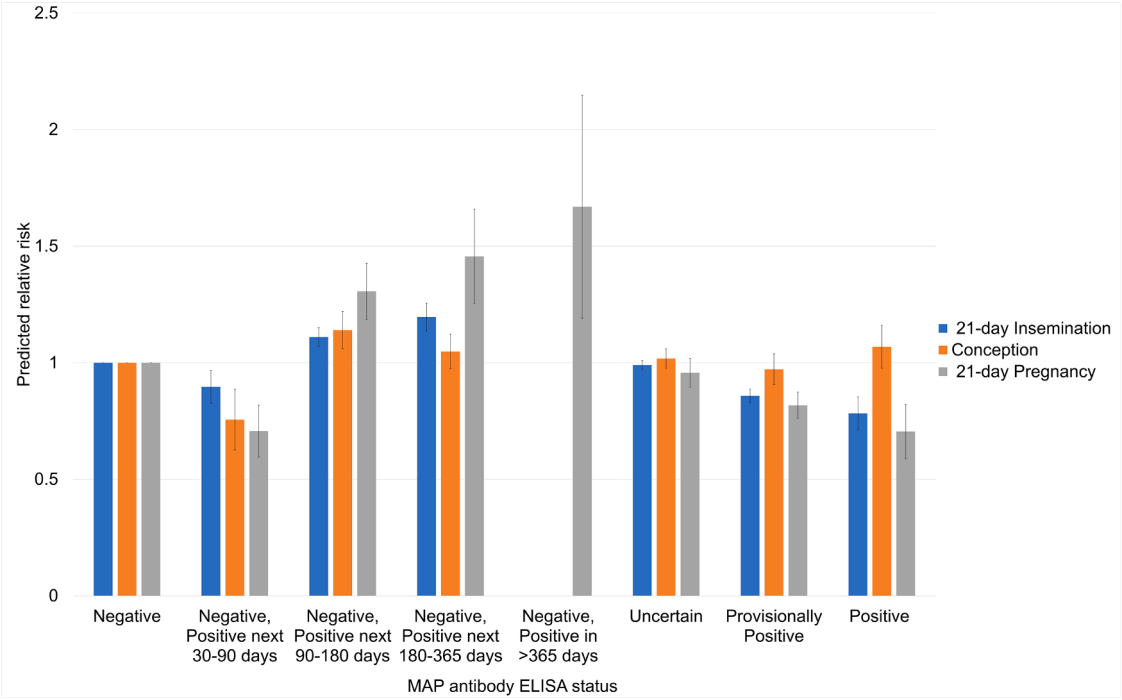

Fig. 2. Association between predicted relative risk of insemination occurring in 21-day risk period, pregnancy occurring in 21-day risk period and conception occurring for given insemination and *Mycobacterium* subspecies *avium paratuberculosis* (MAP) antibody enzyme-linked immunosorbent assay (ELISA) status. MAP antibody ELISA status definitions; negative, currently negative current status; uncertain/provisionally positive/positive, current status; negative, positive next 30–90 days/90–180 days/180–365 days/> 365 days, future non-negative MAP antibody ELISA positive status in 30–90 days/90–180 days/180–365 days/> 365 days after end of risk period (Models 1 and 2) or insemination (Model 3).

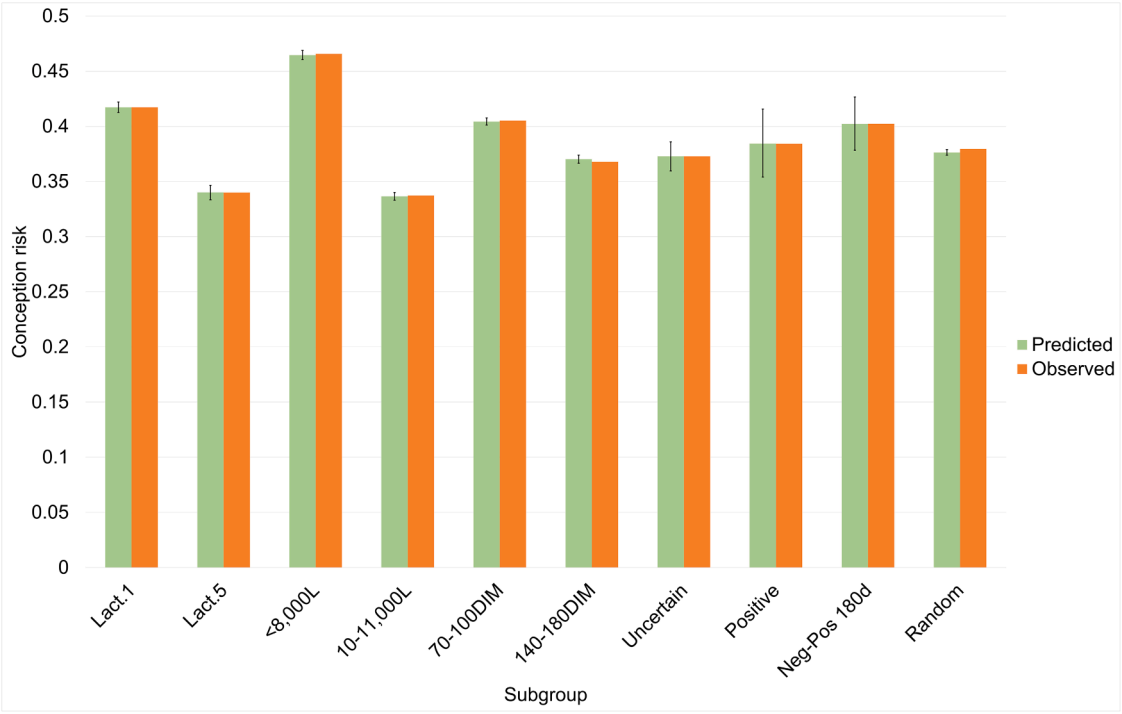

Fig. 3. Conception Risk; Predicted versus observed values by subgroup. Orange bars denote mean observed conception risk, green bars denote median predicted conception risk, error bars denote 95% credible intervals of predicted conception risk. Subgroup definitions: lact 1, all inseminations occurring for lactation 1 cows; lact 5, all inseminations occurring for lactation 5 cows; < 8000 L, all inseminations occurring for cows with 305 day-yield of less than 8000 L; 10–11,000 L, all inseminations occurring for cows with 305 day-yield of between 10,000 L and 11,000 L; 70–100 DIM (days-in-milk), all inseminations occurring between 70 and 100 DIM; 140–180 DIM, all inseminations occurring between 140 and 180 DIM; uncertain, all inseminations occurring for cows with current uncertain *Mycobacterium* subspecies *avium paratuberculosis* (MAP) antibody ELISA status; positive, all inseminations occurring for cows with a current positive MAP antibody ELISA status; neg-pos 180 days, all inseminations occurring for cows that transitioned from negative to non-negative MAP antibody enzyme-linked immunosorbent assay (ELISA) status within next 90–180 days; random, a randomly selected subgroup of 50,000 inseminations.

although this was not statistically significant.

Results from all models are summarised using posterior predictions of relative risk in Fig. 2.

Model checking

All the observed proportion of cases in which a pregnancy or insemination occurred lay within coverage of the 95% credible interval of model predictions across all subgroups for all models, indicating acceptable model fit. Comparison of Model 3 predictions for conception risk with observed mean conception risk for a given insemination across different subgroups is illustrated in Fig. 3.

Discussion

No previous work has robustly evaluated association between fertility outcomes and time to a future positive MAP test status, however this investigation had been recommended in other literature following positive association between positive MAP test status and fertility (Marcé et al., 2009). Trends found in the current study suggest that reduced conception risk for a given insemination is temporally related to occurrence of a positive MAP antibody ELISA result; inseminations recorded closest to a positive MAP antibody ELISA result were less likely to lead to a pregnancy. This is supported by recent UK work which reported that cows with a recent MAP antibody ELISA positive result were more likely to return to service at 56 days and require more inseminations per conception than cows with a less recent positive MAP antibody ELISA result (Pritchard et al., 2017). Previous work also highlighted an increased calving interval during the period of a positive MAP antibody ELISA result (Sibley et al., 2012), although this measures occurrence of insemination as well as conception. Negative energy balance secondary to granulomatous small intestinal inflammation and reduced nutrient absorption efficiency (Sweeney, 2011), has been hypothesised as contributory to reduced conception risk in animals with a positive MAP antibody ELISA status (Johnson-Ifearulundu et al., 2000; Pritchard et al., 2017), however further work is required to verify when this is present relevant to occurrence of a MAP antibody ELISA positive result.

Inseminations associated with a less recent positive MAP antibody ELISA result (Uncertain/Positive status or non-negative transition in more than 90 days), were more likely to lead to a pregnancy. This broadly supports previous work documenting improved fertility in cows with a current Positive MAP antibody ELISA status, although recency of MAP antibody ELISA positive result was not reported in these studies (Lombard et al., 2005; Gonda et al., 2007; Marcé et al., 2009; Smith et al., 2010). Poorer fertility has been demonstrated in high shedding faecal culture positive cows in later stage of disease (Smith et al., 2010) and reducing fertility noted in higher parity ELISA positive animals (Marcé et al., 2009) compared to antibody ELISA negative cattle. The MAP antibody ELISA test was the only diagnostic test used to define MAP test status in this study, however it could be hypothesised that inseminations associated with a less recent positive MAP antibody ELISA result had an increased risk of conception as these animals were not yet in a more advanced state of disease, with reduced fertility associated with disease progression.

It is physiologically unclear why these animals also have a higher probability of conception than Currently Negative animals; a T-helper cell type 2 (T_H2) dominated immune response has been implicated in MAP antibody ELISA positive cows and maintenance of a successful pregnancy (Gonda et al., 2007), however further work is warranted to define a biological mechanism for this and ascertain if this is present in MAP infected cows before first turning ELISA positive.

Both past and current Provisionally Positive and Positive MAP antibody ELISA status were significantly negatively associated with probability of insemination occurring in a 21-day period, consistent with findings in previous work (Raizman et al., 2007). Herd managers will have had access to test results in the current study, declaring cows with more recent or multiple positive MAP antibody ELISA results not to be bred. Transition to any non-negative future MAP test status in the next 90 days was also significantly negatively associated with probability of insemination occurring in a 21-day period, before producers would have been aware of a positive MAP antibody ELISA result. Negative energy balance associated with MAP infection may be implicated in reduced oestrus expression or increased post-partum anoestrus (Butler et al., 2006; Butler, 2003; Johnson-Ifearulundu et al., 2000), although this is currently unconfirmed. Transition to any non-negative future MAP test status in more than 90 days was significantly positively associated with probability of insemination occurring in a 21-day period. Existence of the T_H2 dominated immune response in MAP positive animals is more likely implicated in success and maintenance of conception than oestrus expression or cyclicity, hence the mechanism for this warrants further investigation.

Associations between MAP antibody ELISA status and probability of pregnancy occurring in a 21-day risk period represent a combination of associations between MAP test status and insemination and conception risk.

Data were collected only from larger herds with more accurate records in this study, representing a possible limitation. This makes generalisation of associations between MAP antibody ELISA status and fertility more likely among these types of herd, although biological differences at cow level may be smaller. Furthermore, this type of retrospective study can only establish associations, with further work required to demonstrate causality.

Conclusions

This study demonstrates a largely positive relationship between non-negative MAP antibody ELISA status and cow fertility outcomes prior to a positive MAP antibody ELISA result; negative fertility associations appear leading up to and around the time of a positive MAP antibody ELISA result, with only a long-term negative relationship between insemination risk and non-negative MAP test status thereafter. This demonstrates the potential impact of subclinical MAP infection on dairy cow fertility, amidst a large amount of variability in existing literature and therefore the importance of assessing herd prevalence by regular antibody ELISA testing to help control disease and maximise herd sustainability.

Declaration of Competing Interest

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2023.106015.

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