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MYCOBACTERIUM AVIUM PARATUBERCULOSIS INFECTION OF CALVES – THE IMPACT OF DAM INFECTION STATUS

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ABSTRACT

Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic condition of dairy cattle, and is endemic in the UK. Lack of understanding of the relative importance of different transmission routes reduces the impact of control scheme recommendations. The long incubation period for Johne's disease makes evaluation of control schemes difficult, and so this long-term cohort study offers a rare and valuable insight into the disease epidemiology. A longitudinal study was carried out following a cohort of 440 UK dairy cows in 6 herds recruited in 2012-2013. Individuals entering the milking herd were routinely monitored for the presence of MAP using quarterly milk ELISA testing. Using a Cox proportional-hazards regression model the relationship between time until first detection of infection and dam MAP status was investigated. We then compared the magnitude of the effect of dam status with that of other risk factors in order to understand its relative importance. Dam status was found to be the only observed factor that was significantly associated with time to an individual testing MAP-positive (p=0.012). When compared to negative dams, we found a marginally significant effect of having a positive dam at time of calving, that increased the hazard of an individual testing positive by a factor of 2.6 (95% confidence interval: 0.89-7.79, p=0.081). Further positive associations were found with dams becoming positive *after* the birth of the subject; a dam seroconverting within 12 months post parturition being associated with a 3.6 fold increase in hazard (95% confidence interval: 1.32-9.77, p =0.013), and dams seroconverting more than a year after calving increased the hazard by a factor of 2.8 (95% confidence interval:1.39-5.76, p =0.004). These results suggest that cows may be transmitting MAP to their offspring at an earlier stage than had previously been thought, and so raise important questions about how this transmission may be occurring. The results of the study may have important practical implications for the management on-farm of

the offspring of MAP-positive animals, with the potential to vastly reduce the time required to eliminate this chronic disease.

KEYWORDS

Johne's disease, Mycobacterium avium subspecies paratuberculosis, Control, Dairy Cattle, Herd Health

INTRODUCTION

Understanding the epidemiology of Johne's disease is hampered both by poor diagnostic test sensitivities and by the long incubation period, which lead to slow research progress, and notorious difficulties with control (Dorshorst et al., 2006, Lombard et al., 2005, Meyer et al., 2018). The disease itself is caused by the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP), an intracellular organism affecting the lower small intestine (Whittington, 2010, Harris and Barletta, 2001). Within Great Britain, a cross sectional study has previously estimated the prevalence of MAP-infected herds as ranging between 59% and 77% (Velasova et al., 2017), whilst a separate study in the South West of England has put the proportion of herds with at least a single seropositive animal as high as 75-78% (Woodbine et al., 2009). Initial MAP-infection is believed to be acquired within the first few days of life, but with clinical signs often not appearing until 3-4 years of age (Sweeney, 1996). Such animals continue to deteriorate and will usually be culled on welfare grounds. Further, failure to accurately ascertain the incidence of infection within infected herds is likely to result in underestimation of financial losses associated with both increased culling costs/mortality, and subclinical costs including weight loss, reduced milk yield and poor fertility (Smith et al., 2009).

Transmission of MAP to calves is mainly through ingestion of bacteria, either through the oro-faecal route, or through drinking contaminated milk, though vertical transmission may also play a role (Whittington and Sergeant, 2001, Slana et al., 2008). During the early stages of disease development, infection cannot be detected clinically, neither by faecal nor serological testing. As disease develops, shedding may begin, typically in older youngstock or adult cattle (Mitchell et al., 2011, Nielsen and Ersbøll, 2006). These animals represent an important source of infection to the herd as there may be a large number of such animals, and yet clinical signs are unapparent. From the onset of clinical signs, individuals are likely to be shedding high numbers of MAP in faeces, colostrum, and milk, typically in an intermittent fashion (Whittington and Sergeant, 2001). Clinical signs and high shedding episodes will often be associated with stressful events such as calving, making this a critical period (Martcheva et al., 2015).

Treatment for Johne's disease (JD) is not a viable option, and so herd- level control strategies are based upon prevention of transmission and removal of infectious individuals. Test strategies are now widely adopted in the UK to address these needs (Geraghty et al., 2014). This approach is based upon an indirect ELISA, which can be routinely applied to milk collected as part of individual cow screening. Cows are typically tested on a quarterly basis for JD. Prevention of transmission focuses on the periparturient period, targeting the relationship between the susceptible, new-born calf, and adult animals within the herd. Different management protocols are recommended to reduce new cases of JD within the herd, but detailed information on the relative importance of individual routes of infection are unknown (Geraghty et al., 2014, Garcia and Shalloo, 2015). Whilst culling test-positive cows has been shown to be effective (Collins et al., 2010, Nielsen and Toft, 2011), in practice, known MAP-positive individuals showing no clinical signs are generally retained within the milking herd whilst they remain financially viable, in order to reduce the number of culls carried out. Cows known to be infected will be served to beef bulls, and their offspring reared separately from the milking herd for meat production. However, a significant number of replacement dairy heifers are born to MAP-infected dams, either because they were born prior to detection of MAP, or due to an existing pregnancy at the time of the diagnosis. The full benefit of culling programmes may take many years (Nielsen and Toft, 2011) but better abilities to identify cattle at high risk of being infected may offer possibilities to reduce this time to control.

Nielsen et al. (2016) have shown that calves born to cows identified as positive by milk ELISA at the time of calving and up to 0.7 years later are at higher risk of testing positive themselves. However, Eisenberg et al. (2015a) found no evidence of an association between MAP infection status and the future risk of calves shedding. There is, however, uncertainty as to whether dams in the early stage of Johne's disease pose a risk of transmission of disease to their offspring. This study sets out to investigate the relationship between the dam's MAP status and the likelihood of infection in her offspring. A longitudinal study was carried out, recruiting calves at birth from known JD infected herds allowing comparison of the risk of MAP infection in calves born prior to, and after, the detection of MAP in the dam. The results of this study will be of interest to both farmers and to production animal veterinarians, in guiding their approach to disease management.

MATERIALS AND METHODS

Study herd and animals

During 2012 and 2013, 600 heifer calves were recruited to this study at birth. These animals came from 6 UK dairy herds, of which 2 herds were managed separately on the same holding, so there were 5 different farms included, (the herds are referred to as A-F). All 6 herds were participating in quarterly milk testing of all milking cows, using the IDEXX Porquier ELISA, the most commonly used routine diagnostic test (Nielsen and Toft, 2008), performed by either the National Milk Records (NMR) Group, or the Cattle Information Service (CIS). The incidence rate of new infection was calculated each year for all herds included in order to reflect the likely infection pressure on these farms.

All recruited calves were observed in the calving pen by one of the authors (KB) by using video recording, and an individual calf data capture form was completed relating to the calving process. The following data were recorded for each calf based upon observation of the video recording: cleanliness of the calving yard, number of cows within the calving yard, timing of first colostrum, time the calf remained within the calving pen, and if the calf suckled the dam. These factors were chosen as they are linked to either the likely bacterial burden that the calf would have been exposed to, or the duration of exposure. Cleanliness scores were assigned according to the Wisconsin Hygiene Score (Cook and Reinemann, 2007). The ease of calving (scored as 0 – unassisted, 1 – "easy pull by farmer", 2-"manipulation and pull by farmer", 3-veterinary assisted, and 4- caesarean), source of colostrum (scored as 0-dam, 1-other known cow, 2-pooled, or 3-artificial), quantity of colostrum taken, and the feeding method (scored as 0- bottle, 1- bucket, 2- suckled, or 3-tube fed) were all recorded by the farmer on the data capture form. Ease of calving was included to examine any effects of traumatic birth upon the acquisition of infection. Chest girth was used to determine relative size at birth (Wathes et al., 2008), which is likely to reflect greater quantities of potentially infected colostrum being consumed. A refractometer was used to record colostrum quality (Calloway et al., 2002). The MAP status of the calf's dam was assessed at the point of calving. In accordance with the manner in which the UK dairy industry interprets these results, an ELISA test was considered positive if the sample-to-positive ratio (S/P) was greater than 30%, and inconclusive if the test result was between 20 and 30 % S/P (van Weering et al., 2007). For the purpose of this study, dams were classified as i) Positive, if she had a positive test prior to giving birth to the subject calf, ii) Positive within 12 months, if the first time that she received a positive test result was in the 12 months following the birth of the subject calf, iii) Positive > 12 months, if she tested positive for the first time more than 12 months after giving birth to the subject calf, iv) Inconclusive, if her highest ever test result was between 20 and 30%, or v) Negative, if she scored below 20% S/P on every test during her lifetime.

Calves enrolled in the study were monitored, and following their first calving, were sampled every three months as part of the routine Johne's disease monitoring scheme, with samples for this analysis being collected between June 2014 and March 2017, the study end point. Individuals which were lost from the herd prior to calving, or which were lost prior to the first milk recording point, were excluded from the study. Again, an animal was considered positive from the time point at which it first gave a test result >30% S/P, and these animals were considered as cases for the subsequent analysis.

Data analysis

All analyses performed as part of this investigation were stratified by herd in order to take account of unmeasured differences in management practices. Initially, descriptive statistics to summarise MAP status and frequency of exposure variables across herds were obtained. As part of this initial data exploration, it was assessed whether some factors were too homogeneous within a herd to allow subsequent herd-stratified analyses. Following this step, univariable analyses were carried out, stratified by herd by means of a univariable stratified Cox regression. The time-dependent Cox regression (Cox and Oakes, 1984, van Dijk et al., 2008) was carried out using the Survival package in R (Therneau and Grambsch, 2000). This analysis measured the time from entry into the milking herd until an individual became a case. All explanatory variables were included, individually, in a univariable analysis to investigate their influence upon the hazard.

Finally, a multivariable analysis was performed including any terms for which p<0.2 in the univariable analysis, terms being added in a forward-stepwise process. Models with and without a variable were compared by means of a likelihood ratio test, and the variable retained if p<0.05 (Therneau and Grambsch, 2000). Using the same method, the stratified model was compared to an unstratified version.

RESULTS

Of the 600 enrolled cows, 440 (73.3%) were successfully reared and made it into the milking herds (Table 1). Individual cows were then tested on between 1 and 10 occasions, with a median of 6 tests per cow. By the end of the study period, 55 cows (12.5%) within the cohort had tested positive for MAP at least once, varying from 3% on farm D to 17% on farm F (Table 1). The incidence rate of new infections in the six herds varied over time and between herds, ranging from 0 -14.7 cases/ 100 cows/ year, when taking account of all cows in the herd (both those that formed part of the cohort, and the remaining cows in milk) (Figure 1). Of those heifers born to positive dams, 25.0% tested MAP-positive themselves on at least one occasion. For heifers born to dams that were seronegative at the time of calving and seroconverted later, the proportion was similar: 24.6 % of heifers born to these dams (negative at calving but positive later) tested MAP-positive (Figure 2).

Factors relating to colostrum management (source, quantity, and delivery method) were found not to vary enough within a farm to allow inclusion in the analysis (Supplementary Table 1), these factors were therefore excluded from further analysis. Dam status was analysed as a categorical variable with the original five categories: positive, positive within 12 months, positive more than 12 months after calving, inconclusive, and negative. The distribution of other secondary factors is presented in Table 3.

Univariable analysis of the remaining explanatory variables found dam status to be significantly associated with the hazard of testing positive, when stratifying by herd (p=0.012, Table 4). No other risk factor had a significant association with MAP status and when co-variates were added into the model, no addition made any significant difference to the model. When compared to the unstratified model, using a likelihood ratio test, the stratified model was found to be the better model (p<0.001). The proportional hazards assumption was met for this model (p=0.55).

When compared to negative dams, and stratifying by herd, having a positive dam at the time of calving increased the hazard of testing positive by a factor of 2.6 (95% confidence interval 0.89-7.79, p=0.081). Similar results were obtained for dams that were negative at the time of calving and became positive later: individuals born to dams that tested positive within the first 12 months of their birth had 3.6 times higher hazard of testing positive (95%CI: 1.32-9.77; p =0.013) and those whose dams tested positive more than a year later still had a 2.8 higher hazard of becoming positive (95% CI: 1.39-5.76, p =0.004) than the baseline group of calves born to seronegative dams.

DISCUSSION

The purpose of this study was to investigate the importance of dam status in determining an individual's likelihood of testing positive for MAP, considering not only the status at the time of calving but also future status. Current understanding of Johne's disease transmission is that calves born to MAP-positive dams are at a higher risk of becoming infected, as such dams are expected to be excreting high quantities of MAP in colostrum and faeces which may contaminate the calf during parturition or suckling (Donat et al., 2016). Prior to seroconversion, levels of MAP shedding are assumed to be low (Nielsen and Toft, 2008) and industry guidance in the UK does not make recommendations for the management of calves that are born before a cow first tests positive (AHDB, 2012). However, our findings provide strong evidence that calves are at higher risk of JD even when their dams are negative at the time of calving and seroconvert more than 12 months after the calf's birth.

These findings are strikingly similar to those of Nielsen et al. (2016) who found significant increases in the odds of an individual testing MAP-positive if it was born any time after 8 months prior to its dam testing positive. Eisenberg et al. (2015a, 2015b) however, state that they found no relationship between dam status and offspring shedding. In the latter study, shedding was only monitored in youngstock, and these animals may well have shed later in life. Despite some reported success of culling programmes (Nielsen and Toft, 2011, Strain, 2018), progress is often very slow. Studies showing that test-and-cull strategies alone have a limited impact on the control of Johne's disese (Groenendaal et al., 2002) have recently been challenged by simulation studies that have suggested that these can be more effective (Smith et al. 2017). Including future dam status may be useful to more rapidly remove the offspring of test-positive dams, regardless of the diagnostic timing.

Despite the range of management interventions that are suggested for dairy herds (e.g. (Collins et al., 2010)), the only variable with a significant result in the current study was that for dam status. This is not to say that other interventions do not have an effect. It may just be that the impact of dam status seen here was so large, that the impact of other interventions could not be seen alongside it. These results certainly make a strong case that dam status should be given high importance when determining management practices for Johne's control. It would be interesting to investigate the effect of colostrum feeding practices in more detail, though clearly that was not suited to the current study design. Such practices are likely to be uniformly distributed on most farms, and so a much larger study would be necessary to unpick these effects.

Results from this study appear to be robust, given the study size and the strength of association found. The dam category for cows that were already positive at the time of calving only included 16 individuals and so it is unsurprising that this does not return a significant result. Given the small sample size, the facts that this result does provide weak evidence at all (p=0.081), and that the magnitude of the finding is similar to the other two positive categories, suggests that this finding would be upheld with a larger study. It will be of interest to monitor this population as the study subjects are continually tested. Importantly, these findings are taken from working farms under normal management practices and so are very applicable. The study did not attempt to manage farmers' normal decision making, and herd managers were not blinded to diagnostic test results. Results of the milk-ELISA are commonly interpreted in series, with positive results not being acted upon unless an animal tests positive upon more than one occasion. However, Meyer et al. (2018) have estimated a one-off test specificity of 99.5%, and so for the scope of this study it seems reasonable to consider an animal positive upon the basis of a single positive test. Infection pressures (Figure 1) on the study farms varied, but would appear high enough to suggest that further cases are likely to be found from this cohort. It is unlikely, but possible, that a few subjects may be reclassified as there are a small number of negative dams still in the milking herds that may eventually test MAP-positive. However, these remaining animals are older cows which would have been expected to have seroconverted by this stage. The high degree of similarity between the three categories of positive dam seen in both the hazard ratios (Table 4), and in disease outcome for their offspring (Figure 2) is striking, and of great interest, especially in light of the results of Nielsen et al. (2016). It would be difficult to support such results without a study of this type.

Our study has made use of a long-term dataset to investigate the impact of dam status upon the likelihood of offspring becoming MAP-positive. We have found evidence to support the current understanding that MAP-positive dams are more likely to have MAP-positive offspring than MAP-negative dams, but have also shown in addition that offspring are also more likely to seroconvert if their dam herself seroconverts later in life (i.e. even if they are negative at the time of calving). These findings have interesting management repercussions for dairy farmers, and may explain current difficulties in eliminating Johne's disease from infected herds. The economic implications of altered interventions are, therefore, well worth consideration as a result.

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Table 1 The contribution of each farm to the study. *The number of calves enrolled at each herd is given, together with the number that were successfully reared and entered the milking herd. The final line gives the number of study animals that tested MAP-positive on ELISA on at least one occasion, given as a percentage of animals that reached milking age. Herds A and B were managed on the same premises.*

	A	**B**	**C**	**D**	**E**	**F**	**Total**
Number of cows enrolled	106	121	48	123	144	58	600
Number successfully reared (%)	68 (64.2)	69 (57.0)	33 (68.8)	97 (78.9)	121 (84.0)	52 (89.7)	440 (73.3)
Number testing MAP-Positive (%)	9 (13.2)	6 (8.7)	1 (3.0)	13 (13.4)	17 (14.0)	9 (17.3)	55 (12.5)

Table 2 Distribution of dam statuses. *The Mycobacterium avium paratuberculosis status of the dam of each calf in the study was determined by repeated ELISA. Dams were categorised as Positive if they had received a positive result prior to the calf's birth, Positive within 12 months if they first tested positive within the first 12 months after the birth, or Positive> 12months, if they seroconverted later in life. Dams were classified as inconclusive if their highest ELISA result was between 20 and 30 % S/P. Neg, Neagative; Pos, Positive.*

	Unknown	**Pos**	**Pos within 12m post calving**	**Pos>12m post calving**	**Inconclusive**	**Neg**	**Total**
A	1	3	9	14		41	**68**
B		1		7	4	57	**69**
C	2		1	1		29	**33**
D	5		1	6	1	84	**97**
E		6	7	8	2	98	**121**
F		6	2	9	3	32	**52**
Total	**8**	**16**	**20**	**45**	**10**	**341**	**440**

Table 3 Descriptive Statistics. *The distribution of secondary explanatory variables used in this study (Colostrum feeding factors were not included, and are described separately in Supplementary Tables 1 and 2). Calving figures refer to the farmer-provided description of the ease of calving; Cleanliness is a measure of yard hygiene using the Wisconsin Hygiene Score; Cows in Yard gives the number of cows, other than the dam, in the yard at the time of birth; Suckled Dam and Suckled Other explain the proportion of cattle within each herd that directly suckled their dam, or another cow; Time in pen gives the time spent in the calving yard in minutes; Chest Girth gives the birth size of the calf measured using a calf band; Refractometer Reading gives the quality measure for the colostrum fed.*

	Calving						**Cleanliness**				
	Unassisted	**Easy[1]**	**Farmer Manipulation[1]**	**Vet Calved[1]**	**Caesarean[1]**	**Unknown**	**1**	**2**	**3**	**4**	**Unknown**
A	60	5					6	38	18	3	3
B	67	2					3	37	18	11	
C	29	4					7	22	1	1	2
D	85	7	2					28	60	5	4
E	60	52	7				13	73	22	2	11
F	45	7					4	24	19	1	4
Total	**346**	**77**	**9**	**1**	**1**	**6**	**33**	**222**	**138**	**23**	**24**

[1] These categories were grouped together as "Assisted" for analysis

	Cows in Yard[2]	**Suckled Dam (%)**	**Suckled Other (%)**	**Time in pen[3] (mins)**	**Chest Girth[4] (cm)**	**Refractometer Reading[5]**
	Median (25^{th},75^{th} Percentile)			**Mean (s.d.)**	**Mean (s.d.)**	**Mean (s.d.)**
A	43 (37,50)	90.8	47.7	576.9 (247.4)	74.5 (3.58)	61.7 (6.15)
B	45 (35,51.5)	88.4	49.3	655.0 (310.5)	74.8 (3.81)	62.4 (5.14)
C	3 (2,11)	56.3	12.5	338.3 (543.3)	79.1 (4.10)	58.1 (5.5)
D	21 (20,22)	88.2	46.2	835.1 (527.8)	81.6 (3.49)	61.5 (8.75)
E	6 (5,7)	43.5	15.7	193.6 (175.8)	78.5 (4.86)	58.7 (9.24)
F	17 (16,19)	64.5	22.9	397.5 (284.2)	82.3 (3.66)	54.7 (7.65)

[2] Categorised as 0-30 and 31-60 for analysis. [3]Categorised as <1, 1-5, 5-9, or >9 hours. [4] Categorised as <71, 71-81, or >81 cm. [5]Categorised as <55, 55-62, 62-65, or >65

		Proportion positive	Hazard Ratio	95% Confidence Interval	p	Wald test
Dam Status (n=431)	*Negative*	0.10				**0.012**
	Inconclusive	0.10	1.21	0.16 – 9.24	0.855	
	Positive > 12 month post calving	0.24	**2.83**	**1.39 – 5.76**	**0.004**	
	Positive within 12 month post calving	0.25	**3.58**	**1.32 – 5.77**	**0.013**	
	Positive at calving	0.75	2.63	0.89 – 7.79	0.081	
Calving	*Unassisted*	0.12				0.647
	Assisted	0.15	1.18	0.58 – 2.39		
Cows in yard (n=413)	*Continuous*		1.00	0.96 – 1.05	0.877	0.877
	0-30	0.12				0.566
	31-60	0.24	1.82	0.23 – 14.15	0.566	
Cleanliness (n=415)	*1*	0.09				0.461
	2	0.11	1.18	0.39 – 4.59	0.636	
	3	0.16	1.59	0.52 – 6.68	0.338	
	4	0.04	0.51	0.05 – 5.15	0.577	
Suckled own Dam (n=421)	*No*	0.10				0.356
	Yes	0.12	0.72	0.35 – 1.46	0.356	
Suckled non-Dam (n=421)	*No*	0.12				0.449
	Yes	0.09	0.78	0.42 – 1.48	0.449	
Time to Colostrum n=426)	*Continuous*		1.00	1.00 – 1.00	0.282	0.282
	0-2 hours	0.15				0.790
	2-4 hours	0.11	0.79	0.40 – 1.54	0.485	
	4-6 hours	0.14	1.01	0.45 – 2.26	0.978	
	6-8 hours	0.10	0.60	0.17 – 2.12	0.429	
	>8 hours	0.11	0.51	0.11 – 2.29	0.381	
Time in calving pen (n=426)	*Continuous*		1.00	1.00 – 1.00	0.335	0.335
	< 1 hour	0.14				0.984
	1-5 hours	0.11	0.82	0.27 – 2.51	0.723	
	5 – 9 hours	0.13	0.89	0.26 – 3.06	0.852	
	>9 hours	0.13	0.92	0.27 – 3.18	0.893	
Chest girth cm (n=439)	Continuous		0.99	0.94 – 1.04	0.708	0.708
	<71	0.13				0.455
	71-81	0.11	0.78	0.23 – 2.54	0.667	
	>81	0.15	1.26	0.32 – 3.72	0.880	
Refractometer reading (n=436)	*Continuous*		0.99	0.95 – 1.02	0.421	0.421
	<55	0.14				0.712
	55-62	0.16	1.11	0.55 – 2.25	0.771	
	62-65	0.09	0.71	0.29 – 1.77	0.464	
	>65	0.11	0.82	0.37 – 1.83	0.632	

Table 4 Univariable analysis of the effect of explanatory variables on the time until an individual tests positive for MAP. *Study subjects were subjected to quarterly milk ELISA sampling, and the proportion positive in each category is shown. A univariable Cox-*

proportional hazards regression was carried out stratifying by herd. The final accepted model included only dam status, stratified by herd (Wald test = 0.012, R^2=0.026).

Supplementary Table 1 The distribution of explanatory variables relating to colostrum source and delivery method across the six herds. *The bold figures in brackets indicate the proportion of calves recruited on that farm that received colostrum from that source, or by that method.*

Farm (n)	**Colostrum source**					**Colostrum Delivery method**				
	Dam's colostrum	**Other colostrum**	**Pooled colostrum**	**Powder**	**Unknown**	**Bottle**	**Suckled**	**Tube**	**Bucket**	**Unknown**
A (68)			65 **(0.96)**		3 **(0.04)**	65 **(0.96)**				3 **(0.04)**
B (69)			69 **(1)**			69 **(1)**				
C (33)	32 **(0.97)**	1 **(0.03)**					4 **(0.12)**	27 **(0.82)**	2 **(0.06)**	
D (97)	93 **(0.96)**				4 **(0.04)**		81 **(0.84)**		11 **(0.11)**	5 **(0.05)**
E (121)	117 **(0.97)**	3 **(0.02)**			1 **(0.01)**	117 **(0.97)**		4 **(0.03)**		
F (52)		36 **(0.69)**	**3 (0.06)**	13 **(0.25)**		1 **(0.02)**		51 **0.98)**		
Total (440)	242 **(0.55)**	40 **(0.09)**	137 **(0.31)**	13 **(0.03)**	8 **(0.02)**	252 **(0.57)**	85 **(0.19)**	82 **(0.19)**	13 **(0.03)**	8 **(0.02)**

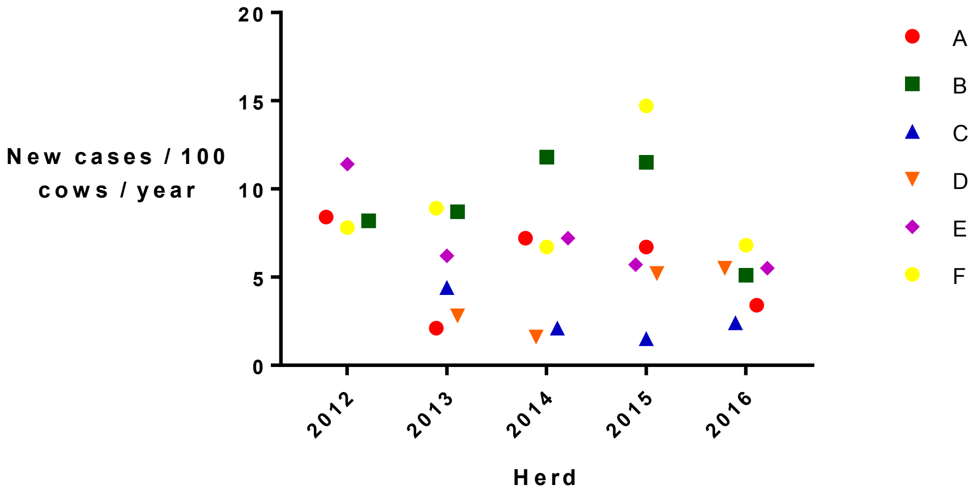

Figure 1 Johne's infection results by herd*. The incidence of new infections is given in each of the study years for each farm. Incidence is expressed as the number of animals testing MAP-positive on ELISA for the first time in a given year, per 100 cows that had never previously tested MAP-positive.*

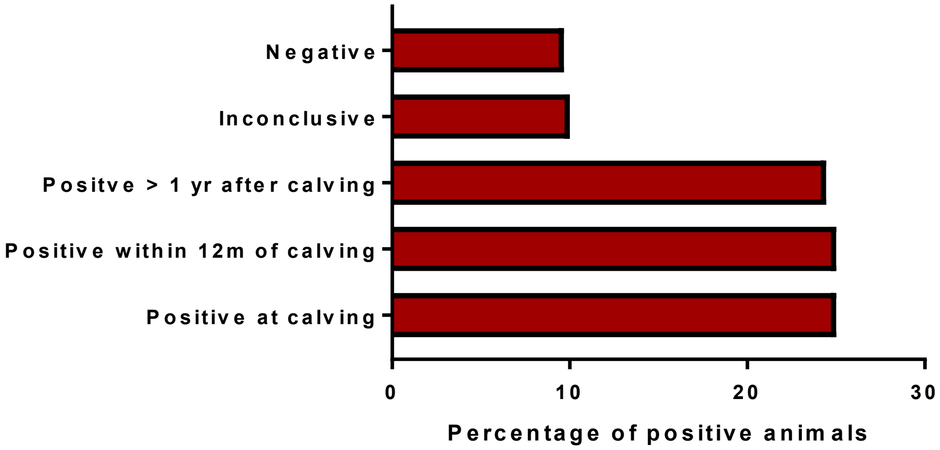

Figure 2 The proportion of study subjects testing MAP-positive in each dam status category. *After entering the milking herd each individual was subjected to repeated milk ELISA tests, and percentages are given for animals testing MAP-positive on at least one occasion, born to dams in different categories across all herds.*