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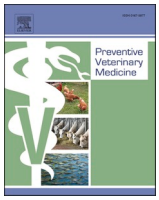

Unravelling transmission of *Mycobacterium avium* subspecies *paratuberculosis* to dairy calves: results of a lifelong longitudinal study

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ABSTRACT

Johne's disease (JD) is a chronic disease of ruminants endemic in the UK and other countries and responsible for large economic losses for the dairy sector. JD is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), which typically infects calves that remain latently infected during a long period, making early detection of infection challenging. Cow to calf transmission can occur in-utero, via milk/colostrum or faecal-orally. Understanding of the different transmission routes to calves is important in informing control recommendations. Our aim in this longitudinal study was to measure the association between the transmission routes via the dam and the environment on a calf subsequently testing serologically positive for MAP. The study population comprised of 439 UK dairy calves from 6 herds enrolled between 2012 and 2013. These calves were followed up from birth until 2023. At birth individual calf data was captured. During follow-up, individuals entering the milking herd were quarterly tested for the presence of MAP antibodies using milk ELISA. Cox regression models were used to measure the association between exposure from the dam (in-utero and/or colostrum) or from the environment (long time in dirty yard) and time to first detection of MAP infection. An association between calves born to positive dams and probability of having a MAP positive test result remained after excluding potential MAP transmission via colostrum (Hazard ratio: 2.24; 95% CI: 1.14 – 4.41). Calves unlikely to be infected with MAP via the in-utero or colostrum route, had 3.68 (95% CI: 3.68 1.45–9.33) higher hazard of a positive test result when they stayed longer in a dirty calving area. The effect of the dam infection status on transmission to calves precedes the dam's seroconversion and persists after excluding the potential role of transmission via colostrum. The association between time spent in a dirty calving area and probability of a MAP positive test result highlights the role of environmental contamination as a source of infection in addition to the dam.

1. Introduction

Johne's disease (JD) is a chronic wasting disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) that affects ruminant populations worldwide and is responsible for a large economic impact for the dairy sector (Hasonova and Pavlik, 2012; Garcia and Shalloo, 2015; Whittington et al., 2019). Furthermore, there is growing concern regarding the public health impact of MAP, with increasing evidence of association between MAP exposure and conditions such as Crohn's disease and multiple sclerosis (Feller et al., 2007; Sechi and Dow, 2015; Ekundayo et al., 2022;). As a result, JD control programs, either voluntary or statutory, are being established in many countries aimed at reducing JD prevalence in dairy herds (Groenendaal et al., 2003; Geraghty et al., 2014). Youngstock are most susceptible to MAP and it is believed that the majority of MAP infections are acquired within the first few days of life (Windsor and Whittington, 2010; Sweeney, 2011; Mortier et al., 2015). Once infected, they may develop clinical disease after an incubation of several years, which coupled with the limited

Abbreviations: DAG, directed acyclic graph; ELISA, enzyme-linked immunosorbent assay; JD, Johne's disease; MAP, Mycobacterium avium subsp. paratuberculosis; S/P, sample-to-positive ratio; UK, United Kingdom.

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ability of diagnostic tests to identify infected animals at early stages of infection (Khol et al., 2011), makes control challenging. Infected animals can shed MAP in faeces, colostrum or milk, which can result in infection of calves through the faecal oral route or through contaminated colostrum or milk (Streeter, and Nielsen et al., 1995, 2008). Infection can also occur "in utero", from infected dam to the foetus, resulting in the birth of an already infected (yet not identifiable) calf (Sweeney, 1996; Whittington and Windsor, 2009). The risk of calf infection is therefore partly driven by the infectious status of the dam (Aly and Thurmond, 2005; Patterson et al., 2020). However, a calf can also be infected via the faecal oral route from faecal contamination originating from cows other than the dam or if given colostrum or milk from cows other than their own dam e.g. if given pooled colostrum (Streeter et al., 1995; Sweeney et al., 1996). Ascertaining the contribution of the dam to the risk of calf infection and the relative contribution of the different infection routes is important to inform disease control measures. However, isolating the effect of the different routes of infection is difficult to achieve from observational studies, as an individual calf tends to be exposed to multiple routes from the dam.

In an effort to gain better insight into the relative role of the different transmission routes, a cohort of 600 heifer calves was recruited at birth during 2012 and 2013 from 6 commercial dairy herds in the UK and followed-up during their entire lives. Previous analysis of this cohort by (Patterson et al., 2020) examined potential risk factors associated with time until MAP positive test result, demonstrating the role MAP status of the dam has on the status of the calf in later life. Importantly, calves born to MAP infected dams were found to have higher risk of MAP infection even when the dam was still testing negative at the time of calving. In the present study, we re-analyse the updated dataset, which now includes records until the end of the follow up for the entire cohort. The main rationale for reanalysis was to allow for more time for seroconversion of infected cows that may have been misclassified as non-infected in the previous analysis due to the chronic nature of the disease. Early results from (Patterson et al., 2020) showed evidence of the dam status as a risk factor for infection, however no evidence of association was found for other potential risk factors at the time when the majority of cows were only in 2–3rd lactation. A potential reason for the apparent lack of other associations could be a difference in time to seroconversion between infection routes.

The aim of the current study was to assess the relative contribution of different MAP transmission routes in dairy cows and examine how time till testing MAP positive was impacted depending on the likely infection route. To this end, the unique set of data gathered during these 11 years allowed testing of a series of hypotheses identified with the help of causal models. We utilised Directed Acyclic Graphs (DAGs) as a tool to visualise the hypothesised casual structure of MAP transmission in calves.

2. Material and methods

2.1. Study design

This was a longitudinal study lasting 11 years to investigate how exposures related to different transmission routes at, or soon after, birth affected the risk of MAP infection in cows belonging to 6 commercial dairy herds in the UK. The present study examined the same cohort as (Patterson et al., 2020) along with updated MAP enzyme-linked immunosorbent assay (ELISA) milk test results for the study animals. The study period was between September 2012 until January 2023. The study animals were all female calves born between 2012 and 2013 in the 6 study farms that entered the milking herds (439 out of 600 female calves born). All 6 study farms had previous evidence of MAP infection based on prior testing and participated in quarterly milk ELISA testing for MAP antibodies. In all of them, environmental factors, colostrum management and dam status were monitored. The 439 study animals were followed up, after their first calving, with quarterly MAP milk ELISA testing. Infected study animals were defined as the subset of study animals with at least one quarterly positive MAP milk ELISA test (IDEXX Porquier ELISA, with a sample-to-positive ratio (S/P) greater than or equal to 0.3). The mean specificity and sensitivity of a single milk ELISA test has been estimated to be 99.5% and 77.5% respectively (Meyer et al., 2018). The methodology of the study has been described previously in (Patterson et al., 2020), where a preliminary analysis of the data was carried out. This study presents the analysis of the final dataset, in which 41 study animals that were considered as non-infected in the previous analysis have been re-categorized as MAP infected following MAP milk ELISA positive test results obtained at late stages of their lives. Only 6 study animals remained alive at the time when the dataset was finalized in January 2023.

2.2. Potential risk factors and transmission routes for MAP infection

At the time of calving, potential risk factors for exposure to MAP were recorded. This included variables related to the farm environment (cleanliness of the calving area), the management of the study animals (colostrum source, length of time the study animals were kept in the calving area) and variables related to the MAP status of the dams and colostrum donor cows (see accompanying Data in Brief for more details on risk factors collected). We define the lifelong MAP infection status of a dam or colostrum donor as positive if they have had at least one positive milk ELISA test result (S/P $\geq$ 0.3) at any point during their lifetime.

Cleanliness of the calving area (measured based on the Wisconsin score for yard cleanliness: 1 = clean to 4 =dirty) and time spent in the calving area were combined into a new binary variable: 'long time in dirty yard'. Study animals spending more than the median time for the cohort (429 min) in a calving area that had a score greater than 2 were defined as study animals that stayed longer in a dirty calving area.

Study animals received colostrum by 4 different methods: own dam, donor cow, pooled or artificial. During the study period, quarterly MAP test results of the dams and any donor cow that gave colostrum to a study animal were obtained, allowing definition of exposures based the MAP status of the dam or the donor. Identification of the cows contributing to pooled colostrum was not feasible, therefore it was not possible to trace back the MAP status for the cows contributing to pooled colostrum. Artificial colostrum was assumed to be negative for MAP.

We examined 3 specific potential routes of MAP transmission in calves: in-utero, milk/colostrum and environmental, with the statistical models to be used for each being informed by DAGs presenting the underlying causal assumptions. DAGs are sets of nodes connected by edges (graphs) in which connections have direction (directed) and in which for a given node, if the edge connecting it to another node is followed, there is no path to return to the initial node (acyclic) (Greenland et al., 1999; Pearl, 2009; Hernan and Robins, 2017). DAGs can be used to characterise causal associations between exposures and outcomes with the presence of an arrow direct from one variable to another variable denoting the direct causal relationship between the two variables and by including in the DAG all common causes of the exposure and outcome (confounding). A square box around a node represents restriction to a certain level of the node (also called conditioning). DAGs can be used to strengthen causal inference in observational studies addressing potential sources of bias in exposure-outcome relationships (Hernan and Robins, 2017). DAGs are well established in human epidemiology but so far had limited adoption in the context of animal health and veterinary epidemiology. Figs. 1, 2 and 3 present three different DAGs representing three sub-studies aimed at examining the different infection routes. In all three cases we assume that a cow that tested positive at any point in its lifetime was infected at the time of calving. This assumption seems reasonable given that MAP infects cattle at early stages of their lives (Windsor et al., 2010) and noting the high specificity (99.5%) of the MAP antibody milk ELISA (Meyer et al., 2019).

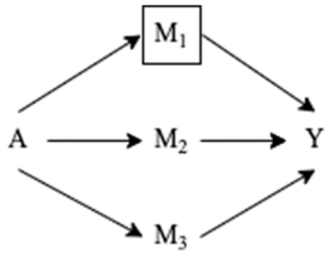

Fig. 1. Direct Acyclic Graph (DAG) representing a cohort study with an exposure variable (A): lifelong MAP infection status of the dam and an outcome variable (Y): Study animals MAP infection status. Exposure (A) is partially mediated by M1 (transmission via colostrum from the infected dam), M2 (in utero transmission from the infected dam) and M3 (transmission through environmental contamination from the infected dam). The study assesses the association between A and Y through routes other than colostrum, therefore conditioning on M1 through restriction i.e. only study animals that were given colostrum from a negative source were included.

Fig. 2. Directed Acyclic Graph (DAG) representing a cohort study with an exposure variable (A): being fed colostrum from a cow positive to MAP, an outcome variable (Y): Study animals MAP infection status and a confounder (X): lifelong MAP infection status of the dam. A square around X represents conditioning on status of the dam. The study assesses the association between A and Y conditioning on X through restriction i.e. only study animals born to non-infected dams were included.

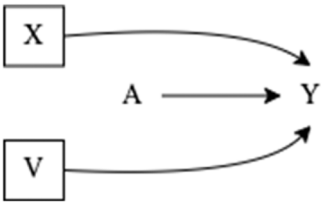

Fig. 3. Directed Acyclic Graph (DAG) representing a cohort study with an exposure variable (A): staying longer in a dirty calving area and an outcome variable (Y): study animals MAP infection status. Variable X represents lifelong MAP infection status of the dam and variable V represents lifelong MAP infection status of the cow (or donor dam) source of colostrum. Both these variables are likely to be associated with Y. Restriction on X and V whereby only study animals born to non-infected dams and not given colostrum from infected cows were included.

2.3. Statistical analysis

Descriptive statistics were obtained for all variables under study. The Cox proportional hazards model used to examine the overall effect of lifelong MAP infection status of the dam in the previous study by (Patterson et al., 2020) was rerun with the updated status of study animals.

Cox proportional hazards models were used to assess the causal associations between exposure variables at time of calving and time until first evidence of infection in study animals (first positive MAP milk ELISA result). Three separate models were built to assess the associations of interest in accordance with the DAGs presented in Figs. 1, 2 and 3.

Model 1. : The relationship between lifelong MAP infection status of the dam and MAP status of study animals through routes other than colostrum (i.e. in utero or environmental from their own dam) was assessed with dam status as the only explanatory variable and including only study animals that were given colostrum from a presumed negative cow (pooled colostrum was assumed to be negative in this model; Fig. 1).

Model 2. : The relationship between being fed colostrum from a MAP positive cow and MAP positive study animals (i.e. colostrum route) was assessed with MAP status of the cow (donor or dam) giving colostrum as the only explanatory variable. Including only study animals born to non-infected dams (Fig. 2).

Model 3. : The relationship between spending a long time in a dirty yard and MAP infection of the study animals (i.e. environmental route from any cow) was assessed with the variable "staying longer in a dirty calving area" as the only explanatory variable and including only study animals born to non-infected dams and given colostrum from a non-infected cow (Fig. 3).

In all models, study animals contributed to follow up time from the date of first MAP antibody milk ELISA test to occurrence of MAP positive test result, culling date or end of study (6 cows), whichever occurred first. All models were stratified by farm.

Time to a MAP antibody positive test result was compared between two groups of infected study animals that tested positive during the study period:

i) Infected study animals born to positive dams and given colostrum from a known positive source (these cows may have been infected in utero or from colostrum or milk).
ii) Infected study animals born to a negative dam and that were not given colostrum from a positive cow (these study animals can be assumed to be infected through the faecal oral route and as a result of contamination from cows other than the dam).

Kaplan Meier curves for time to a MAP positive test result were compared for the two groups by means of Tarone-Ware test. Statistical models were implemented in Python 3.8 using the package lifelines (Davidson-Pilon, 2019).

3. Results

Assuming infected study animals that tested positive at least once in their lives were MAP infected and that the testing regime did not miss any infected study animals, the proportion of MAP-infected study animals ranged between 16.5% and 28.8% across the 6 study herds. Out of the 439 study animals successfully entering milking herds as adult cows, 96 (21.9%) tested positive at least once since the start of the study. Median time to a positive test result was 682 days (IQR 273–985). Descriptive statistics for the study variables are presented in Table 1. Overall, 28% of the infected study animals were not exposed to any of the risk factors under consideration (i.e. they were not born to MAP positive dams, not exposed to MAP via colostrum and not held for a prolonged period in a dirty calving area).

Results from the Cox proportional hazards model used by Patterson et al. (2020) to assess the overall effect of MAP status of the dam on calves becoming MAP positive, rerun with updated MAP status of study animals, resulted in a hazard ratio of 1.89 (95% CI: 1.18–3.03).

The results of the three Cox proportional hazards models corresponding to the three sub-studies addressing the different transmission routes (Figs. 1, 2 and 3) are presented in Table 2. The results of model 1 show that study animals born to MAP positive dams and not given colostrum from positive cows had 2.24 (95% CI: 1.14–4.41) higher hazard of testing positive to MAP than study animals born to negative dams and not receiving colostrum from positive cows. This hazard ratio therefore represents the combined estimated effects of in-utero transmission and transmission through the environment due to their animals' own dams. Model 2 assesses the effect of receiving colostrum from a MAP positive cow among study animals born to a negative dam. The

Table 1
Descriptive statistics for outcome (testing positive against MAP) and exposure variables in 439 study animals from 6 UK dairy herds subject to quarterly testing for the presence of MAP antibodies in milk since entering the milking herd until censoring (culling / end of study) or till event.

		N	Positive[a] (%)
Herd	A	70	14 (20.0)
	B	67	17 (25.4)
	C	32	8 (25.0)
	D	97	16 (16.5)
	E	121	26 (21.5)
	F	52	15 (28.8)
Dam status	Negative	353	67 (19.0)
	Positive before birth	14	3 (21.4)
	Positive after birth	72	26 (36.1)
Colostrum status	Negative	232	44 (19.0)
	Positive	59	18 (30.5)
Colostrum source	Dam	242	48 (19.8)
	Other cow	40	11 (27.5)
	Pooled	137	32 (23.3)
	Artificial	13	5 (38.5)
Stayed longer in a dirty calving area[b]	No	145	32 (22.1)
	Yes	105	23 (21.9)

[a] Study animal tests positive by MAP antibody milk ELISA at least once.
[b] Study animal spent longer than median time for the cohort (429 min) in a yard that had a Wisconsin score greater than 2.

Table 2
Results of three separate stratified Cox proportional hazards models examining associations between exposures representing different MAP transmission routes and time until MAP positive status (results from a cohort study of 439 dairy cows from 6 UK dairy herds).

		N	Positive (%)	HR (95% CI)	p value
Dam status[a]	Negative	339	63 (18.5)	1	
	Positive	41	15 (36.6)	2.24 (1.14–4.41)	0.01
Colostrum status[b]	Negative	232	44 (19.1)	1	
	Positive	14	4 (28.6)	1.64 (0.52–5.17)	0.40
Spent long time in dirty yard[c]	No	166	27 (16.5)	1	
	Yes	54	15 (28.8)	3.68 (1.45–9.33)	0.01

[a] Analysis restricted to cows not given colostrum from MAP positive cows.
[b] Analysis restricted to cows born to MAP negative dams.
[c] Analysis restricted to cows born to MAP negative dams and not given colostrum from MAP positive cows.

estimated hazard ratio was 1.64 (95% CI: 0.52–5.17), hence the model does not provide evidence of an association between being given colostrum from a positive dam and MAP status, but only 14 study animals were given colostrum from a positive cow when restricting analysis to study animals born to MAP negative dams, which reflects in the wide confidence interval. The results from model 3 provide evidence of an association between spending long time in a dirty yard and MAP positive status. Study animals that stayed longer in a dirty calving area were 3.7 times more likely (95% CI: 1.45–9.33) to test MAP positive compared to study animals that did not, when restricting the analysis to cows born to MAP negative dams and that did not receive colostrum from positive dams.

Kaplan Meier curves for time until MAP positive milk ELISA test result in infected study animals born to MAP positive dams or given colostrum from a MAP positive cow (dam or colostrum route) and calves born to MAP non-infected dams and not given colostrum from a known positive cow (environmental route) are presented in Fig. 4. The median survival time (time to a MAP positive test result) was 429 days for cows in the first group (those potentially exposed to MAP in-utero and/or through colostrum or milk) and 642 days for cows in the second group (those not exposed to MAP in utero or through colostrum/milk). Despite the difference between the two groups being large, they are based on small numbers (only 11 cows in the first group) and the results do not provide clear evidence of an effect of the potential route of infection on time until positive result (P = 0.18, by Tarone Ware test).

4. Discussion

Control of JD in dairy herds remains a priority and a challenge for the dairy industry globally. In addition to the impact of JD on cattle productivity and welfare, effective JD control is becoming a pressing need in light of growing evidence linking MAP and human health (Sechi et al., 2015). Farm-level control measures are directed towards interrupting the main transmission routes: faecal oral infection, infection from milk or colostrum and in-utero transmission (Nielsen, and Streeter et al., 2008, 1995). Their simultaneous occurrence makes it difficult to ascertain their relative role, which is important to inform farm-level control practices. Furthermore, due to the chronic nature of the disease and low sensitivity of tests at early stages of infection, long-duration studies are needed to ascertain association with infection.

In this study we have addressed the two challenges above, first by following a cohort of female cows through their entire lives, including regular testing of their dams and secondly by identifying suitable comparisons within a cohort framework to disentangle concurrent exposures and assess their effects on risk of becoming MAP positive. As for the first point, the importance of conducting a sufficiently long study is reflected in the time needed for animals to seroconvert. Four years after the study begun only 55% of cows that eventually tested positive had been identified and 80% after 6 years. As for the second issue, explicit consideration of potential biases and causal relationships, based on our assumptions laid out in the DAGs, allowed us to carry out comparisons within the cohort that with adequate conditioning (by means of restriction) minimized risk of bias. These comparisons provided evidence of the causal effect of each route on the risk of calves becoming MAP positive.

Our results provide strong evidence that JD infection risk is partly driven by the status of the dam through infection routes different to colostrum/milk, most likely as a result of in-utero infection. Importantly, the risk posed by an infected dam (determined by testing) is not limited to those identified as infected at the time of calving. This finding, which was reported in our previous study (Patterson et al., 2020), is important as it shows that strategies based on limiting replacement from cows already found to be positive will have limited effect.

Environmental contamination from cows other than the dam also plays a role in infection risk, as shown by the strong effect of keeping calves borne to negative dams and given colostrum from known negative cows for a long time in a dirty calving area. This finding is in agreement with the results of previous studies of the association between management related risk factors and MAP infection (Berghaus et al., 2005). In 2018, Benedictus et al. showed how a calving pen contaminated by MAP-shedding animals results in a higher risk of MAP infection in calves born to lifelong test negative dams.

We were unable to identify an association between MAP infection status and being given colostrum from an infected dam or donor. However, this finding should be interpreted with caution given the small numbers available to assess this association after restricting the analysis to cows born to negative dams (only 14 cows born to negative dams where given colostrum from a positive donor cow). An additional challenge in assessing the impact of the colostrum route is that MAP excretion is likely to be associated with stage of infection, with cows at early stages of MAP infection unlikely to shed MAP in colostrum (Sweeney et al., 1992). This further compromises the capacity of our study to assess the role of the colostrum route. A further difficulty of assessing the role of the colostrum route is the potential for MAP being present in colostrum as a result of contamination with faeces containing MAP. Other studies have tried to determine if ingesting MAP positive

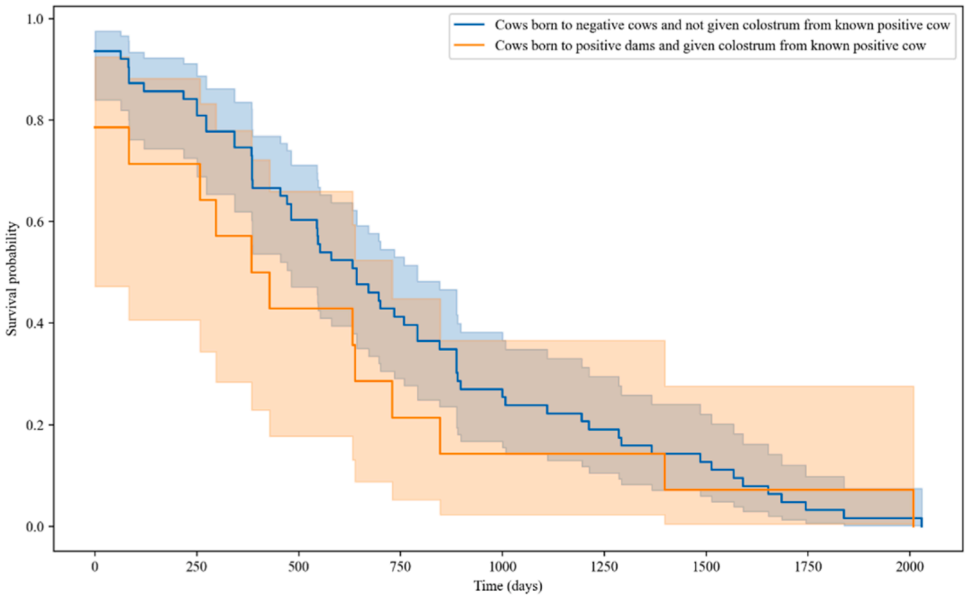

Fig. 4. Kaplan Meier survival curves representing time till positive MAP test result from first test date for two groups of cows: orange line represents infected study animals born to positive dams and given colostrum from positive cows (n = 11); blue line represents infected study animals born to negative cows and not given colostrum from positive cows (n = 60). Shaded areas represent 95% confidence intervals.

colostrum leads to infection, however disentangling this from infection via other routes remains a challenge (Nielsen et al., 2008; Pithua et al., 2011).

With regard to potential differences in time to testing positive depending on the potential infection route, although with considerable uncertainty, the identification of a large apparent difference between the groups warrants further research. Small numbers, particularly for the subgroup born to a positive dam or fed colostrum from a positive donor cow, results in large uncertainty around time till MAP positive test result for this subgroup. Previous studies have suggested a dose response relationship between MAP intake and time till being detected as positive for MAP (Benedictus et al., 2008). Our results are compatible with calves infected via dam-daughter routes experiencing a higher dose of MAP compared to cows born to negative dams that become infected via the environment, but this remains speculative. Our study has some limitations. We are defining as outcome a positive test in MAP milk ELISA. For purpose of disease control, more stringent definitions are often used but given the high specificity that has been reported for the test (Meyer et al., 2019) it was reasonable to assume cows testing positive were identified correctly. As explained above, sample size has compromised to some extent our ability to estimate the effect of some exposures due to restriction of the cohort.

Through lifelong follow up of a well-characterized cohort of dairy cows, we have been able to ascertain the effect of specific exposures on the risk of MAP infection. Our results strongly suggest that in a given infected farm, new infections are a result of a combination of in-utero and faecal-oral infections, with infection from colostrum being a possibility but challenging to be quantified and separated from other infection routes. The relative contribution of the dam vs. other cows to the maintenance of MAP infection will be higher in farms with good hygienic management of newborn calves. Although our results show associations between status of the dam and time spent in a dirty calving area with MAP status, a considerable proportion of animals were deemed infected without being exposed to any of the risk factors examined, which suggests other unknown exposures also contribute to MAP transmission.

In conclusion, this study provides evidence of MAP infection risk being partly but not entirely driven by the dam's infection status. Notably, the dam's impact persists even when removing potential transmission via colostrum. Time spent in dirty calving also contributes to risk of MAP infection. The findings of this study highlight the difficulties in control as the proportion of MAP-positive dams detected before calving was small. Additionally, a considerable proportion of infected animals had not been found to be exposed to the specific risk factors under investigation. These findings underscore the need for improved methods of early detection and a broader understanding of alternative routes of transmission to effectively control MAP infection.

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Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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References

Aly, S., Thurmond, M., 2005. Evaluation of Mycobacterium avium subsp paratuberculosis infection of dairy cows attributable to infection status of the dam. J. Am. Vet. Med. Assoc. 227, 450–454. https://doi.org/10.2460/javma.2005.227.450.

Benedictus, A., Mitchell, R., Linde-Widmann, M., Sweeney, R., Fyock, T., Schukken, Y., Whitlock, R., 2008. Transmission parameters of Mycobacterium avium subspecies paratuberculosis infections in a dairy herd going through a control program. Prev. Vet. Med. 83, 215–227. https://doi.org/10.1016/j.prevetmed.2007.07.008.

Davidson-Pilon, C., 2019. lifelines: survival analysis in Python. J. Open Source Softw. 4, 1317. https://doi.org/10.21105/joss.01317.

Ekundayo, T., Olasehinde, T., Falade, A., Adewoyin, M., Iwu, C., Igere, B., Ijabadeniyi, O., 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.

Feller, M., Huwiler, K., Stephan, R., Altpeter, E., Shang, A., Furrer, H., Pfyffer, G., Jemmi, T., Baumgartner, A., Egger, M., 2007. Mycobacterium avium subspecies

paratuberculosis and Crohn's disease: a systematic review and meta-analysis. Lancet Infect. Dis. 7, 607–613. https://doi.org/10.1016/S1473-3099(07)70211-6.
Garcia, A.B., Shalloo, L., 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.
Geraghty, T., Graham, D., Mullowney, P., More, S., 2014. A review of bovine Johne's disease control activities in 6 endemically infected countries. Prev. Vet. Med. 116, 1–11. https://doi.org/10.1016/j.prevetmed.2014.06.003.
Greenland, S., Pearl, J., Robins, J.M., 1999. Causal diagrams for epidemiologic research. Epidemiology 10, 37–48. https://doi.org/10.1097/00001648-199901000-00008.
Groenendaal, H., Nielen, M., Hesselink, J., 2003. Development of the Dutch Johne's disease control program supported by a simulation model. Prev. Vet. Med. 60, 69–90. https://doi.org/10.1016/S0167-5877(03)00083-7.
Hasonova, L., Pavlik, I., 2012. Economic impact of paratuberculosis in dairy cattle herds: a review. Vet. Med. 51, 193–211. https://doi.org/10.17221/5539-VETMED.
Hernan, M.A., Robins, J.M., 2017. Causal Inference. Chapman & Hall/CRC,, Florida.
Khol, J., Geisbauer, E., Wassertheurer, M., Revilla-Fernández, S., Damoser, J., Österreicher, E., Dünser, M., Kleb, U., Baumgartner, W., 2011. Outcome of three commercial serum ELISAs and faecal detection of Mycobacterium avium subsp. paratuberculosis in consecutive samples from a cattle herd with low prevalence of paratuberculosis (Johne's Disease). Transbound. Emerg. Dis. 59, 197–207. https://doi.org/10.1111/j.1865-1682.2011.01256.x.
Mortier, R., Barkema, H., De Buck, J., 2015. Susceptibility to and diagnosis of Mycobacterium avium subspecies paratuberculosis infection in dairy calves: a review (https://doi.org). Prev. Vet. Med. 121, 189–198. https://doi.org/10.1016/j.prevetmed.2015.08.011.
Nielsen, S., Bjerre, H., Toft, N., 2008. Colostrum and milk as risk factors for infection with Mycobacterium avium subspecies paratuberculosis in Dairy Cattle. J. Dairy Sci. 91, 4610–4615. https://doi.org/10.3168/jds.2008-1272.
Patterson, S., Bond, K., Green, M., Winden, S. van, Guitian, J., 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.
Pearl, J., 2009. Causal inference in statistics: an overview. Stat. Surv. 3. https://doi.org/10.1214/09-ss057.
Pithua, P., Godden, S., Wells, S., Stabel, J., 2011. Evaluation of the risk of paratuberculosis in adult cows fed Mycobacterium avium subs paratuberculosis DNA-positive or -negative colostrum as calves. Am. J. Vet. Res. 72, 1456–1464. https://doi.org/10.2460/ajvr.72.11.1456.
Sechi, L., Dow, C., 2015. Mycobacterium avium ss. paratuberculosis Zoonosis – The Hundred Year War – Beyond Crohn's disease. Front. Immunol. 6. https://doi.org/10.3389/fimmu.2015.00096.
Streeter, R.N., Hoffsis, G.F., Bech-Nielsen, S., Shulaw, W.P., Rings, D.M., 1995. Isolation of Mycobacterium paratuberculosis from colostrum and milk of subclinically infected cows. Am. J. Vet. Res. 56, 1322–1324. https://doi.org/10.1128/aem.64.9.3153-3158.1998.
Sweeney, R., 1996. Transmission of Paratuberculosis. Vet. Clin. North Am. Food Anim. 12, 305–312. https://doi.org/10.1016/s0749-0720(15)30408-4.
Sweeney, R., 2011. Pathogenesis of paratuberculosis. Vet. Clin. North Am. Food Anim. 27, 537–546. https://doi.org/10.1016/j.cvfa.2011.07.001.
Sweeney, R., Whitlock, R., Rosenberger, A., 1992. Mycobacterium paratuberculosis cultured from milk and supramammary lymph nodes of infected asymptomatic cows. J. Clin. Microbiol. 30, 166–171. https://doi.org/10.1128/jcm.30.1.166-171.1992.
Whittington, R., Windsor, P., 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.
Whittington, R., Donat, K., Weber, M.F., Kelton, D., Nielsen, S.S., Eisenberg, S., Arrigoni, N., Juste, R., Sáez, J.L., Dhand, N., Santi, A., Michel, A., Barkema, H., Kralik, P., Kostoulas, P., Citer, L., Griffin, F., Barwell, R., Moreira, M.A.S., Slana, I., Koehler, H., Singh, S.V., Yoo, H.S., Chávez-Gris, G., Goodridge, A., Ocepek, M., Garrido, J., Stevenson, K., Collins, M., Alonso, B., Cirone, K., Paolicchi, F., Gavey, L., Rahman, M.T., de Marchin, E., Van, W., Bauman, C., Fecteau, G., McKenna, S., Salgado, M., Fernández-Silva, J., Dziedzinska, R., Echeverría, G., Seppänen, J., Thibault, V., Fridriksdottir, V., Derakhshandeh, A., Haghkhah, M., Ruocco, L., Kawaji, S., Momotani, E., Heuer, C., Norton, S., Cadmus, S., Agdestein, A., Kampen, A., Szteyn, J., Frössling, J., Schwan, E., Caldow, G., Strain, S., Carter, M., Wells, S., Munyeme, M., Wolf, R., Gurung, R., Verdugo, C., Fourichon, C., Yamamoto, T., Thapaliya, S., Di Labio, E., Ekgatat, M., Gil, A., Alesandre, A.N., Piaggio, J., Suanes, A., de Waard, J.H., 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.
Windsor, P., Whittington, R., 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.