Detection of a high prevalence of paratuberculosis in a previously test-negative conventional dairy herd in the Netherlands

David C. Speksnijder1,2 | Mirthe K. de Wit3 | Betsie Krattley-Roodenburg1

1 University Farm Animal Clinic, Harmelen, The Netherlands
2 Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
3 Student at Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract

Johne's disease, or paratuberculosis, in cattle, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), can cause substantial economic losses on dairy farms. This case study describes the finding of a MAP prevalence of 8% in a Dutch dairy herd with a 10-year history of biennial enzyme-linked immunosorbent assay-negative testing in individual milk samples of lactating cows. This case illustrates the strengths and limitations of the different laboratory tests and testing schemes for MAP screening in dairy herds. A cluster of MAP-shedding cattle on this farm was related to a birth cohort, which suggests a common source of infection early in life. Our observations stress the importance of preventive management to reduce the potential and hidden spread of MAP in test-negative herds.

**KEYWORDS**

dairy cattle, disease control, paratuberculosis

**BACKGROUND**

Paratuberculosis (Johne's disease) in dairy cattle, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), can cause substantial economic losses due to decreased milk yield and feed conversion efficiency, emaciation leading to death and premature culling.1,2 There is a substantial body of evidence that suggests a link between Johne's disease in cattle and Crohn's disease in humans, although this link remains under ongoing scientific debate. As MAP survives normal pasteurisation procedures and viable bacteria can enter the human gut through normal milk consumption, MAP is regarded by many countries as a food safety issue. This disease is therefore subject to active control policies in different countries, varying from prevalence reduction to attempts to completely eradicate the disease.1–4

MAP infections in cattle typically occur during the first months of life, although there is no strict age resistance. The most important infection route is the faecal–oral route, although transmission via colostrum or in utero is reported.2,5 The epidemiology of paratuberculosis in cattle is complex, as clinical disease only becomes visible in latter stages of disease and diagnosis is challenging due to the low sensitivity of available tests.2 This demands a long-term commitment of the complete dairy industry in order to control the disease.3

The current nationwide control policy for Johne's disease in the Netherlands was launched in 2000 by the Dutch dairy industry, aiming to reduce the concentration of MAP in bulk milk delivered to the milk factories.6,7 The MAP control activities in the Netherlands started as a voluntary programme based on individual faecal culture, but a mandatory control policy based on antibody testing (ELISA), came into force for all dairy farmers in 2010 as part of the Dutch Milk Quality Assurance Programme (MQAP).8 This control policy consists of biennial ELISA antibody testing for MAP in individually derived milk samples from lactating dairy cows and confirmation of positive animals by bacteriological culture or PCR on faecal samples, followed by mandatory culling of PCR-confirmed cases.8,9 Additionally, it is recommended to cull offspring under the age of 1 year of dams confirmed positive with PCR. Dry cows are not tested in this Dutch MAP control strategy. In 2019, 77% of the Dutch dairy farms had paratuberculosis status 'A', which means that no ELISA-positive lactating cow has been found in the last biennial test event among lactating animals in the herd and no other MAP-positive animal has been diagnosed since this test event.10 Data from a cohort study on 551 Dutch dairy herds indicated that 57% of these farms retained status A for at least 10 years' and the longer they retained status A the lower the probability of losing status A due to the detection of a MAP-positive animal.11 A recent analysis on Dutch data showed that the cumulative probability of losing status A is around or below 0.2, 6–10 years after achieving this status, implying that losing status A is not common once a farm has secured status A for a couple of years.12
In this case report, we present the unexpected discovery of a high MAP prevalence in a long-term antibody test-negative dairy herd to show the complex epidemiology of paratuberculosis in dairy farming and the necessity to take continuous precautionary measures, even on MAP test-negative farms.

CASE PRESENTATION

An unexpectedly high prevalence of paratuberculosis without clinical signs was detected in a dairy herd of approximately 180 adult cattle and 150 youngstock in 2019, although a couple of known risk factors for introduction and spread of paratuberculosis were present. Adult dairy cows on this farm were housed in a freestall with cubicles and were grazed during the summer months. Youngstock were housed in a separate barn until they reached approximately 18 months of age. The herd was not closed. Between 2008 and 2012, no animals from other farms were introduced. However, between 2013 and 2018, a total of 53 animals were purchased from five different herds. In 2013, two suckling calves and 21 heifers (between 9 months and 2 years of age) were purchased from three different herds, although these were not always the herd of birth of these animals. In 2015, five pregnant heifers were purchased from one farm but these heifers had all different origin farms. In 2018, 25 adult cows had been introduced from a farm that received status A in 2016 but had an origin of having MAP-seropositive cows. Additionally, in the summer of 2017, a total of 79 homebred youngstock were temporarily grazed on pastures of two other farms in the neighbourhood. Calvings mostly occurred in a separate calving pen, but young calves were not always separated from the dam directly after birth. The hygiene of the straw bedding material in the calving pen had not always been optimal over the last couple of years according to the farmer. The calves were fed colostrum from their own dam in the first 2 days after birth, followed by milk replacer during the rest of the suckling period. Occasionally, pooled colostrum and bulk milk were fed to calves in the last years, including the replacement heifers. Before and after weaning, the heifers received the farm’s own harvest grass silage and special concentrate for calves. The calves were housed individually for the first weeks of life and thereafter in small groups on straw-bedded pack until the age of 5 months when they were moved to a freestall with cubicles.

INVESTIGATIONS

Between 2008 and 2018, milk samples of all lactating cows on this farm were tested every other year by the Dutch Animal Health Service (Royal GD, Deventer, the Netherlands) with the Pourquier (currently IDEXX) Paratuberculosis Screening Ab Test as part of the active surveillance system for controlling Johne’s disease within the Dutch MQAP (Figure 1). This test uses an elevated cutoff S/P ratio of 1.0 in order to increase the diagnostic specificity, which is, however, dependent on the MAP prevalence within a herd. Under Dutch circumstances, the probability of a positive faecal culture result of milk Ab ELISA-positive animals is reported to be approximately 0.7 using this S/P ratio. Until 2016, only negative results were obtained from this farm. In August 2018, three out of 132 tested lactating cows had a positive milk Ab ELISA result. MAP infection was confirmed by positive faecal qPCR results in two of these three cows, born in 2011 and 2012 and homebred (index cases). Following these MAP-positive results, both cows were culled. The cow that tested negative (born in 2015) was removed from the herd, which is in accordance with the regulations of the Dutch MQAP.

In April and May 2019, as a voluntary follow-up and as replacement of the Ab screening of the lactating cows, all cattle ≥12 months of age were tested by individual qPCR on individual faecal samples. In March 2020 and April 2021, all animals older than 2 years were retested by qPCR on individual faecal samples. In 2020, all offspring older than 12 months of dams with a positive qPCR result were tested with qPCR on pooled (five samples/pool) faecal samples.

OUTCOME AND FOLLOW-UP

Out of the 253 tested animals in April/May 2019, 20 animals had a positive qPCR test result (8% of the tested animals) (Table 1). Six of these animals had a negative milk Ab ELISA result in August 2018, the others had not been tested in 2018, because they were either dried off or still a heifer at that moment. All 20 MAP-positive animals were homebred and born between January 2010 and July 2017, except one cow that was introduced from another herd at the age of 4 years in June 2018. This herd of origin received status A in 2016, but had seropositive animals in 2015 (status B). A basic univariate analysis based on test outcome did not indicate any significant association of the category of animal (homebred, temporarily raised in another herd, or introduced from another herd) or age category (1, 2, 3 or ≥4 years of age) and qPCR result. However, a cluster of qPCR-positive homebred heifers born in 2017 became apparent: seven out of 12 homebred heifers born in April and May 2017 appeared qPCR positive. These heifers had never left the premises. The dam of the oldest of these 12 heifers had tested qPCR positive in April 2019 and would be a plausible source of infection of these heifers. Four other dams, which were still present on the farm, had tested qPCR negative in April 2019. Of these four negative dams, one tested
positive in March 2020, two others tested negative again and the remaining cows had been culled by the time of the second testing. Also a heifer born in July 2017 tested positive in May 2019 while its mother tested negative on qPCR both in 2019 and 2020.

In March 2020, 11 animals out of 181 cattle above 2 years tested positive on individual qPCR (6% of the tested herd). All positive cows where homebred and born between 2013 and 2016 at the time of testing, although one animal was born in 2018 (25 months old). Three of these animals had been temporarily raised on another farm as a heifer. One animal (born in 2016) from a dam that had tested qPCR positive at the same sampling date in 2020, but not in 2019. Another animal (born in 2016) tested negative in 2019 but was born from a dam that tested qPCR positive in 2019. From these 11 positive cows, five female calves >1 and <2 years were present on the farm. These have been tested in April 2020 using qPCR on pooled faeces but tested negative.
In April 2021, three animals out of 174 above 2 years tested positive on individual qPCR (2%). These animals were born in 2012, 2015 and 2019, all homebred and none had been raised elsewhere. Neither dams nor offspring of these animals had tested positive as far as they were present.

All positive tested animals were culled within a few months after testing qPCR positive. If offspring from these positive animals was of <1 year of age, these calves were sold for slaughter according to the general recommendations. The retained cow that tested positive in the Ab test in 2018 tested PCR negative in the respective investigations in 2018, 2019 and 2020 and was removed from the herd in 2021.

After the discovery of the unexpected high number of MAP-positive animals in 2019, preventive measures for calves up to 6 months of age were advised to the farmer. The advice related to housing, included to design sufficient calving pens that allowed easy cleaning and disinfecting after each calving, to remove replacement heifers immediately after birth and to wear separate boots and coveralls in the calf rearing area. Recommendations related to feeding included to exclusively feed calves colostrum from their own dam followed by milk replacer and to feed calves grass silage harvested from pastures that were not fertilised with manure from adult dairy cows. Additionally, it was advised to cull calves from the cluster of 12 heifers with a high prevalence of MAP found in 2019 and to refrain from introducing cattle from other farms.

**DISCUSSION**

In this case herd, in total 34 animals tested qPCR MAP positive on faecal samples within a period of 2 years, after 8 years of biennial serological MAP-negative testing in individual milk samples of lactating cows.

The aim of the Dutch MQAP is to reduce the concentration of MAP in bulk milk and to provide assurance regarding milk quality in certified test-negative herds. For this purpose, herds do not necessarily need to be free of MAP infection, which is also often echoed in the communication to farmers. This farm received the status A classification in 2008, but it is obvious that this herd was not truly MAP negative at that time. Ten animals that were later detected with MAP were born between 2011 and 2013, before the farm started to introduce new animals to the herd, indicating that at least 10 animals with MAP had been present in the herd already 5–7 years while being undetected. Finding a relatively large cluster of MAP-positive heifers is rather unusual. A recent study showed that the age of becoming milk Ab ELISA positive in Dutch dairy farms has increased over the last decade, which is an indication of the effectiveness of the Dutch MAP control strategy and the decreasing prevalence of MAP.13,14 The cluster of MAP-positive young animals that was found on this farm indicates that there has been a high infection pressure on this farm in 2017 as the age of onset of ELISA positivity is related to the infectious dose and age at the time of infection.12

This case confirms that the milk Ab ELISA test is able to detect the presence of MAP on farms with a ‘high’ prevalence of MAP-positive cows (>5% PCR positive), although the sensitivity at individual cow level is very low.14 In this case study, only two qPCR-positive cows had been detected with the milk Ab ELISA, while six other qPCR-positive cows in April 2019 tested milk Ab ELISA negative 8 months earlier. Several explanations exist for this finding. There has been a gap of several months between the individual milk Ab ELISA testing and the qPCR screening on faeces. It is possible that these six lactating cows just recently had started to excrete MAP bacteria in their faeces and seroconverted, but it is highly unlikely that this is true for all these animals. Recent findings indicate that the milk Ab ELISA should ideally not be used as a binary presence/absence of infection indicator at individual cow level, which is very much dependent on the time of sampling related to the stage of the infection and the chosen cut-off value.13,15 Another explanation could be that the majority of the qPCR-positive cattle on this farm were non-progressive or transient shedders, of which it is known that the probability of testing ELISA positive is relatively low at the time when faecal culture or qPCR on faeces becomes positive, although it is questionable whether this holds true for six out of eight qPCR-positive animals that tested milk Ab ELISA negative in 2018.15,16

This case shows that if farmers aim to use test and cull as part of their strategy to control MAP infections in their herds as much as possible, it is important to consider that faecal qPCR has a considerably higher diagnostic sensitivity to detect individual MAP-shedding cattle and that a considerable proportion of infected cattle start shedding MAP before or shortly after reaching adulthood—as illustrated by the observations in this case herd. It also shows the added value of performing further diagnostics on all animals above 12 months after the detection of an antibody-positive MAP animal in a herd to specifically detect MAP-shedding animals that can be removed from the herd as part of the strategy to control paratuberculosis on the farm. Although the serum Ab ELISA has a slightly higher sensitivity compared to the milk Ab ELISA, qPCR on faecal samples of faecal culture is preferred because of its higher sensitivity.12,14 Taking serum samples of dry cows in addition to the milk Ab ELISA testing of lactating cows to get the full picture of the MAP Ab status of all adult cows could however be a low-cost addition to the regular testing scheme. It should be stressed, however, that the sensitivity of all the available tests, including qPCR, is far from perfect and that testing alone does not replace the importance of other control factors on farm.

A cluster of MAP-shedding cattle on this farm could be related to birth cohort. Potential transmission routes were a contaminated calving pen, transmission through feeding of colostrum and bulk milk from multiple cows to calves and calf-to-calf transmission. Our observations stress the importance of taking preventive management measures to reduce the potential and hidden spread of MAP in test-negative herds as well.

**ACKNOWLEDGEMENT**

We thank Dr. Maarten Weber for his valuable input regarding the used test methods and their characteristics at Royal GD, the Netherlands.

**FUNDING INFORMATION**

The authors received no specific funding for this work.

**CONFLICT OF INTEREST**

The authors declare they have no conflicts of interest.
ETHICS STATEMENT
This study described a case that occurred in the field. The diagnostic approach, outcomes and follow-up are part of the usual veterinary care in the Netherlands and complied to the existing paratuberculosis control programme in place. No ethical approval was therefore necessary.

REFERENCES

How to cite this article: Speksnijder DC, deWit MK, Krattley–Roodenburg B. Detection of a high prevalence of paratuberculosis in a previously test-negative conventional dairy herd in the Netherlands. Vet Rec Case Rep. 2022;10:e290. https://doi.org/10.1002/vrc2.290