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SHORT COMMUNICATION

Mycobacterium avium subspecies *paratuberculosis* in pooled faeces and dust from the housing environment of herds infected with Johne's disease

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD) in ruminants characterised by a long incubation period where infected animals progressively excrete MAP in their faeces with some animals, 'super shedders', dispersing large quantities of the organism into their environment where it can survive for long periods,.[1 2] Control programmes for the disease primarily focus on the early detection and removal of shedding animals from herds, appropriate calf management practices, good herd biosecurity and hygiene practices together with measures preventing spread of infection within or between herds.[3] Environmental contamination with MAP and its role as a source of infection is well recognised,.[4–6] More recently concerns have been raised that MAP persisting in dust in cow barns and in areas from which it is difficult to remove[7] may become airborne and a source of infection for animals through respiratory uptake.[8 9]

A JD pilot control programme was initiated in Ireland in 2013 and follows international best practices.[10] This study aims to support these best practices and examines the dispersion of MAP in the environment of infected dairy herds with special focus on its recovery from dust in barns and milking parlours.

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Five herds (A–E), confirmed as heavily infected in the preceding 4 to 10 years before sampling, were selected. The herds applied different control measures but the data collected over the years indicated that at least 10 per cent of animals in these herds were confirmed to be shedding MAP at some point with some 'super shedders' also present. Culturing of faecal samples in the months before environmental sampling showed that 45/115 of the animals sampled in herd A, 9/345 in herd B, 1/65 in herd C, 0/28 in herd D and 11/213 in herd E were shedding MAP with one high shedder present in herd A, one moderate shedder present in both herds B and E, and one low shedding animal in herd C. Herd D had no shedders recorded at the time of environmental sampling.

All the farms were visited between October 2017 and March 2018. Pooled environmental faecal samples were also collected using individual spoons from high traffic area of the farm including passageways in sheds and the milking parlour collecting yards. Settled dust samples were collected from indoor locations including walls/ridges/ledges in barns and milking parlours, overhanging support bars for milking equipment or feeding troughs where present using a whirl pak hydrated speci-sponge (Nasco, USA).

Faecal samples were cultured using the Cornell double incubation decontamination method.[11] Following manufacturer's instructions they were also tested by direct qPCR as described elsewhere,[11] targeting the IS900 sequence using the LSI VetMAX kit (LSI, Lissieu, France) and the spin column Qiagen DNA mini kit (Qiagen, Manchester, UK). Dust samples were stomached in sterile DNA/RNA-free water and the supernatant transferred to a sterile tube and allowed to settle for 30 minutes followed by culture and direct qPCR testing. In addition, all the dust liquid culture

Table 1 Culture and qPCR results from environmental pooled faeces and dust samples collected from five herds infected with Johne's disease. MAP, *Mycobacterium avium* subspecies *paratuberculosis*.

Herd	Years since first MAP infection confirmed in herds	Animals in herd, n	Pooled faeces No positive/no samples (%)		Dust samples No positive/no samples (%)	
			Culture	IS900 qPCR	Culture	IS900 qPCR
A	4	251	10/10 (100)	9/10 (90.0)	0/10 (0)	9/10 (90)
B	4	340	9/13 (69.2)	7/13 (53.8)	0/11 (0)	6/11 (54.5)
C	6	90	1/10 (10)	1/10 (10)	0/10 (0)	0/10 (0)
D	7	62	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)
E	10	464	4/10 (40)	6/10 (60)	0/10 (0)	2/10 (20)

media were subjected to direct qPCR and F57 qPCR screening after their culture period was completed,[12] using the extraction as described above.

Culture confirmed MAP in four herds; the organism cultured from 45.3 per cent (n=24) of the pooled faecal samples while direct faecal qPCR gave positive MAP signals in 43.4 per cent (n=23) of the same samples (table 1).

The number of positive samples varied between herds, being highest in herd A where the highest number of shedding animals (n=45) was recorded at the time of environmental sampling. The absence of MAP from the pooled environmental faecal samples in herd D was not surprising as this herd had made extensive efforts in recent years, through a combination of testing and improved herd management practice, to remove all positive or suspect animals from the herd. Test agreement for culture and qPCR for pooled faecal samples had a kappa value of 0.65 (95 per cent CI 45.2 to 86.0) and the overall test agreement was calculated at 83 per cent.

Of the 51 dust environmental samples collected, 17 (33.3 per cent) samples from three herds (A, B, E) were positive on direct qPCR but none were positive for MAP on culture. The most common sources for qPCR-positive dust environmental samples were the surfaces of support bars (80 per cent) in milking parlours and sheds, and the feeding bins in herd A (table 2).

Direct qPCR on the culture media showed that 11 (21.6 per cent) samples gave positive signals on IS900 qPCR but none on the F57 qPCR. Of these, eight (72.7 per cent) were positive on direct IS900 qPCR when applied directly to both dust and dust culture media. Positive IS900 PCR results in the absence of MAP culture and F57 qPCR confirmation assay most likely indicate the presence of mycobacterial DNA but not viable cells. The isolation of MAP from samples is regarded as the 'gold standard' for confirmation of its presence,[13] but as the culture process included a decontamination step which is detrimental to MAP survival and growth,[14] it is possible that samples with low MAP numbers may be recorded as negative. In addition, the intermittent shedding of MAP by some animals could have also resulted in some animals being missed at the time of sampling. MAP dormancy has been documented,[1] and accumulated dormant MAP cells away from high traffic within the barns may also have accounted for some culture negative results. Pretreatment and extraction procedures required in the qPCR assay may also have affected the qPCR results, and an additional limitation of the qPCR testing is its inability to distinguish between viable and not viable MAP cells,.[15] While the IS900 sequence is considered highly sensitive and specific for MAP, a positive signal has also been reported from environmental mycobacteria.[11 16 17] Although less sensitive, the single-copy F57 sequence in MAP genome is currently considered as the most specific target for MAP confirmation.[12 18]

While the finding of positive signals for MAP in direct qPCR in dust samples collected from barns and milking parlours including the feeding bins in this study is a concern, the results do not indicate that viable MAP were present. However, preventing soiling of feeders is important, and MAP DNA presence is indicative of poor application of the hygiene measures essential for the control of MAP. Our results indicate that dust is not a significant source of infection for animals. Dust screening may also be less effective than pooled environmental faecal screening for the detection of MAP-positive dairy herds.

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Competing interests None declared.

Table 2 Results of MAP culture and PCR testing of dust and of PCR on the post culture media from the samples collected from various housing sites from five farms. MAP, *Mycobacterium avium* subspecies *paratuberculosis*.

Sample location	Dust samples collected, n	Positive dust samples, n		Positive culture media samples on PCR, n	
		IS900 qPCR (%)	Culture (%)	IS900 qPCR (%)	F57 qPCR (%)
Support bars	10	8 (80)	0 (0.0)	4 (40)	0 (0.0)
Feeders/feeding traps	4	3 (75)	0 (0.0)	1 (25.0)	0 (0.0)
Barn walls/ledges	37	6 (16.2)	0 (0.0)	6 (16.2)	0 (0.0)
Total	51	17 (33.3)	0 (0.0)	11 (21.6)	0 (0.0)

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