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Presence of *Mycobacterium avium* subsp. *paratuberculosis* in Environmental Samples Collected on Commercial Dutch Dairy Farms[▽]

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***Mycobacterium avium* subsp. *paratuberculosis*, the causative agent of Johne's disease in cattle, was identified in settled-dust samples of Dutch commercial dairy farms, both in the dairy barn and in the young stock housing. Bioaerosols may play a role in within-farm *M. avium* subsp. *paratuberculosis* transmission.**

Paratuberculosis is an infectious enteric disease caused by *Mycobacterium avium* subsp. *paratuberculosis* leading to economic losses in dairy cattle globally (2, 10). The main transmission route is the fecal/oral route from infectious adult cattle to susceptible calves (12).

Preventive calf management was a key point in model studies (7), but 20-year implementation did not lead to farm-level eradication, suggesting uncontrolled routes of transmission (1, 7).

Environmental samples were used to classify commercial dairy herds (3, 9, 11), based on long-term survival of *M. avium* subsp. *paratuberculosis* in the environment (16). Recently, bioaerosols containing viable *M. avium* subsp. *paratuberculosis* were identified in an experimental setting with 100% *M. avium* subsp. *paratuberculosis* prevalence (6) and may thus be a mode of transmission. Dust containing *M. avium* subsp. *paratuberculosis* might be ingested or inhaled by calves (4). Experimental *M. avium* subsp. *paratuberculosis* challenge studies in sheep successfully used inhalation (8). These transmission routes could hamper current control programs. Our objective was to study whether *M. avium* subsp. *paratuberculosis* could be detected in bioaerosols on commercial Dutch dairy farms.

Dairy herds in three Dutch veterinary practices were sampled in 2009. All farms participated in a Dutch *M. avium* subsp. *paratuberculosis* monitoring program in 2008, either the Dutch Paratuberculosis Program (PPN; $n = 2$) or the Dutch Bulk Milk Quality Assurance Program (BMQAP; $n = 22$) (15). Both PPN herds were certified *M. avium* subsp. *paratuberculosis*-free. Herds corresponding to the BMQAP had at least one positive animal identified by enzyme-linked immunosorbent assay (ELISA) (Pourquier ELISA; Institut Pourquier, France). Farms were grouped into three *M. avium* subsp. *paratuberculosis* test prevalence levels (control, zero positive animals; group A, one positive animal; group B, two or more positive animals; Table 1).

Farms were visited twice during the housing period. Sampling locations were above the animal level inside the barn. At the first visit (sampling 1 [S1]), settled dust was collected with wipes and a short management questionnaire was taken. At the same time, five to seven electrostatic dust collectors (EDC; Zeeman, Alphen a/d Rhijn, Netherlands) were installed and collected after 4 weeks (sampling 2 [S2]) (6). Settled-dust samples were processed according to a previously described method (6). Results are presented as proportions of positive locations. McNemar's χ^2 test was performed to investigate whether S1 differed from S2.

No *M. avium* subsp. *paratuberculosis* was detected by real-time PCR in any of the settled-dust samples at control farms (Fig. 1). *M. avium* subsp. *paratuberculosis* DNA was detected in dust samples at S1 and S2 in more than 50% of the group A and B farms, with seven farms consistently positive. *M. avium* subsp. *paratuberculosis* DNA was detected in the young stock area in 3/6 (S1) and 2/6 (S2) farms of group B with single-barn housing. *M. avium* subsp. *paratuberculosis* DNA was also detected in settled-dust samples from separate young stock housings in three farms, of which two cohoused dry cows.

At control farms, no viable *M. avium* subsp. *paratuberculosis* was detected in any of the collected dust samples (Fig. 2). Viable *M. avium* subsp. *paratuberculosis* was detected in 6 B farms at S1. At S2, viable bacteria were present in 3 A farms and in the majority of B farms (Table 2). On five farms in group B, viable *M. avium* subsp. *paratuberculosis* was detected at both samplings.

Viable *M. avium* subsp. *paratuberculosis* was detected in the young stock housing in 4 and 3 farms of group B with single-barn housing at S1 and S2, respectively. No viable *M. avium* subsp. *paratuberculosis* was detected in separate young stock housings.

To our knowledge, this study is the first to confirm the presence of *M. avium* subsp. *paratuberculosis* DNA as well as viable *M. avium* subsp. *paratuberculosis* in settled-dust samples of commercial dairy farms. *M. avium* subsp. *paratuberculosis* dispersion by bioaerosols under experimental conditions was

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TABLE 1. Overview of the results of the questionnaire about relevant *M. avium* subsp. *paratuberculosis* management practices[a]

Parameter	Value for group[b]		
	Control (n = 2)	A (n = 8)	B (n = 14)
Mean herd size (SD)	69 (15)	67 (19)	102 (26)
Median no. of ELISA-positive cows (maximum)	0 (0)	1 (1)	3 (10)
No. of farms with:			
Cow brush in barn	2	5	13
Cow barn cleaned in summer with high-pressure cleaner	0	6	4
Dry cows in young stock housing	0	3	3
Young stock housed separately	1	7	8
Young stock housing empty in summer	0	0	0
Young stock housing cleaned with high-pressure cleaner	0	6	1

[a] Results of the questionnaire about relevant *M. avium* subsp. *paratuberculosis* management practices in 24 Dutch farms enrolled in this study with 0 (control), 1 (group A), or ≥2 (group B) ELISA-positive animals.
[b] n, number of farms.

already described (6). These findings support the concept of dust-based environmental dispersion of *M. avium* subsp. *paratuberculosis* within farms.

The relatively small number of farms and the convenience sampling are limitations of this study that could have introduced bias. However, this study is a proof of principle that viable *M. avium* subsp. *paratuberculosis* can be detected in settled-dust samples on farms with a low *M. avium* subsp. *paratuberculosis* prevalence. The environmental method also seems specific for *M. avium* subsp. *paratuberculosis*, since no *M. avium* subsp. *paratuberculosis* could be detected in any samples of known *M. avium* subsp. *paratuberculosis*-free herds.

Paratuberculosis control measures aim to prevent fecal-oral contact between infectious shedding adults and susceptible

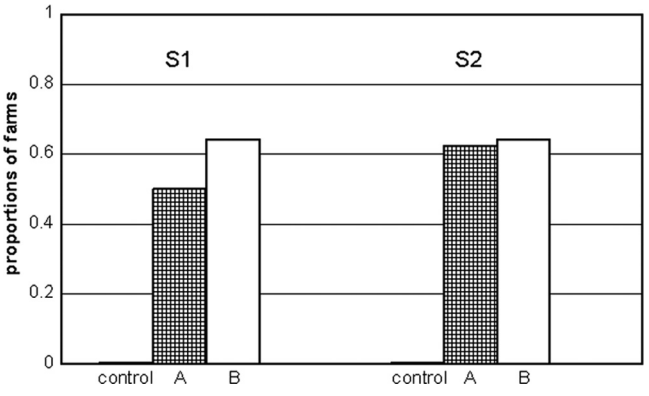

FIG. 1. Proportions of farms with *M. avium* subsp. *paratuberculosis* DNA detected in settled-dust samples collected at samplings 1 and 2. Black bar, control (n = 2); checked bar, group A (n = 8); white bar, group B (n = 14).

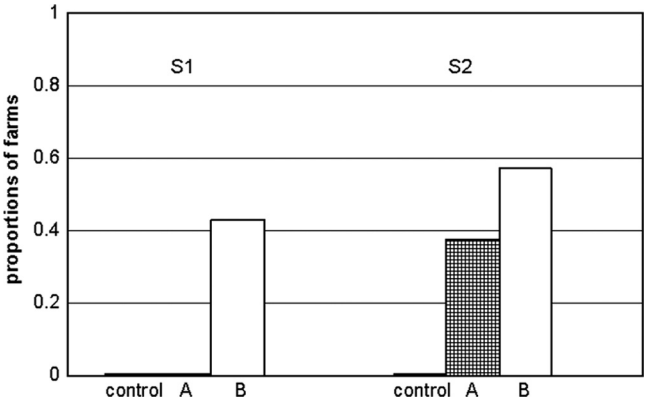

FIG. 2. Proportions of farms with viable *M. avium* subsp. *paratuberculosis* detected in settled-dust samples collected at samplings 1 and 2. Black bar, control (n = 2); checked bar, group A (n = 8); white bar, group B (n = 14).

calves as the main transmission route of *M. avium* subsp. *paratuberculosis*. Several studies showed that "calf hygiene improvement" decreased prevalence but did not eliminate the disease (1, 7, 14), suggesting the existence of other transmission routes. *In utero* transmission, transmission via milk, and calf-to-calf transmission have been described previously (1, 12, 13). Additionally, infection via ingestion and/or inhalation of bioaerosols may be possible (4, 8).

Twenty-three of 24 herds were housed in free stalls with one tie-stall herd. Most farmers (n = 15) separated young stock from adult cattle as standard procedure. However, six of these farmers cohoused dry cows in the young stock housing occasionally, indicating the difficulties of consequently implementing management advice. Three farmers did not raise young stock on their farms. In almost all barns, cow brushes were present, as they were recommended to enhance cow well-being in group housings (5), but at the same time they contribute to aerosolization of dust. Animal movement on slatted floors also contributes to dust formation, especially during the winter housing period.

Most farmers from group A farms, compared to only a few from group B farms, intended to clean their barns yearly, but only 50% met this aim. Young stock housings were never totally empty, but high-pressure cleaning was occasionally performed at 6/8 farms of group A and at 1 of group B. The numbers of farms in this study precluded statistical testing, but the difference in cleaning attitude seemed remarkable.

Comparison of the two methods of dust collection showed no statistical difference. No *M. avium* subsp. *paratuberculosis*, neither DNA nor viable *M. avium* subsp. *paratuberculosis*, could be detected on known negative farms, whereas on farms of groups A and B, *M. avium* subsp. *paratuberculosis* DNA was present in comparable numbers of locations. Viable *M. avium* subsp. *paratuberculosis* was present only in group B farms at S1 and in both group A and B farms at S2. It seems that *M. avium* subsp. *paratuberculosis* can survive in dust for some time. Besides having a possible role in *M. avium* subsp. *paratuberculosis* transmission, dust might also be a useful predictor of *M. avium* subsp. *paratuberculosis* presence or *M. avium* subsp. *paratuber-*

TABLE 2. Detection of *M. avium* subsp. *paratuberculosis* DNA or viable *M. avium* subsp. *paratuberculosis* in 5 to 7 settled-dust samples collected at sampling 1 or 2

No. of positive dust samples	No. of farms with:											
	M. avium subsp. *paratuberculosis* DNA						Viable *M. avium* subsp. *paratuberculosis*					
	Control (n = 2)		Group A (n = 8)		Group B (n = 14)		Control (n = 2)		Group A (n = 8)		Group B (n = 14)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
0	2	2	4	3	4	5	2	2	8	5	8	6
1			3	4	4	6				1	2	4
2					4	3				1	1	2
3			1	1	1	1				1	1	2
4					1						2	

culosis introduction on dairy farms, even on farms with low *M. avium* subsp. *paratuberculosis* prevalence.

In conclusion, this study showed that dust on farms with a low *M. avium* subsp. *paratuberculosis* seroprevalence contained viable *M. avium* subsp. *paratuberculosis*, which indicated a role in *M. avium* subsp. *paratuberculosis* transmission. Further research is needed to study if and how infection with *M. avium* subsp. *paratuberculosis*-contaminated dust is possible. Additionally, dust sampling may be an alternative tool to monitor *M. avium* subsp. *paratuberculosis* status in control programs.

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