

TABLE S1 Characteristics and management practices of the investigated dairy farms

Factor	Farm A	Farm B	Farm C
Herd size	20	40	60
Milking system	Parlour	Parlour	Automatic
Barn structure	Open air	Heat-insulated	Heat-insulated
Housing system	Bedded pack	Free-stall	Free-stall
Bedding material	Peat, straw	Peat	Sawdust, sand
Stall mats	NA ^a	Rubber mat/pad	Pasture gel mat
Manure passage type	Concrete	grid	concrete
Feces removal system	shovel	mechanic scrape	mechanic scrape
Roughage feeding system	feed table	feed table	feed troughs
Farming system	organic	conventional	conventional

^aNA, not applicable; the resting area is not divided into stalls

TABLE S2 Proportion of *Listeria monocytogenes* positive 50 ml-aliquots in 250-ml composite bulk tank milk samples collected from farms A–C.

Proportion of positive aliquots	MPN [CI 95%] ^a (cfu/ml)	Farm A		Farm B		Farm C	
		n ^b	% ^c	n	%	n	%
0/5	0 [0–0.03]	63	84	51	86	47	90
1/5	0.01 [0.001–0.07]	8	11	4	7	4	8
2/5	0.02 [0.005–0.03]	0	0	4	7	1	2
3/5	0.04 [0.01–0.1]	2	3	0	0	0	0
4/5	0.07 [0.02–0.2]	1	1	0	0	0	0
5/5	> 0.07 [NA ^d]	1	1	0	0	0	0

^a MPN [CI 95%]: Most probable number of *L. monocytogenes* in the sampled bulk tank milk and 95 % confidence limits, derived from the number of aliquots positive for *L. monocytogenes* after enrichment

^b n: number of composite bulk tank milk samples

^c %: percentage of composite bulk tank milk samples

^d NA: not applicable; the confidence limit is incalculable

TABLE S3 Comparison of hygiene scores and *L. monocytogenes* prevalences on farms A–C. For each evaluated and sampled site, the three farms were ranked based on hygiene scores and *L. monocytogenes* prevalence: the highest hygiene score was ranked first, and the lowest hygiene score was ranked third; the lowest prevalence was ranked first, and the highest prevalence was ranked third

Evaluated site	Sampled site	Farm A		Farm B		Farm C	
		HS (R) ^a	% (R) ^b	HS (R)	% (R)	HS (R)	% (R)
Milk room	Milk room floor	3 (1)	36 (1)	3 (1)	45 (1)	2.5 (3)	46 (3)
Milking station	Milking station floor	1 (3)	58 (2)	3 (1)	26 (1)	2 (2)	69 (3)
Waiting area	Waiting area floor	1 (3)	64 (2)	3 (1)	36 (1)	1.5 (2)	67 (3)
Resting area	Bedding	2 (3)	38 (3)	3 (1)	5 (1)	3 (1)	15 (2)
Cow cleanliness	Udder surface	2 (3)	31 (3)	3 (1)	4 (2)	3 (1)	0 (1)
Feed troughs	Feed trough surface	1.5 (3)	31 (3)	2 (2)	24 (2)	3 (1)	16 (1)
Water troughs	Water trough surface	2 (3)	29 (2)	3 (1)	19 (1)	3 (1)	31 (3)
Total score	Environmental prevalence	1.7 (3)	21 (3)	2.8 (1)	10 (1)	2.6 (2)	17 (2)

^a HS (R): Hygiene score and ranking

^b % (R): *L. monocytogenes* prevalence and ranking

TABLE S4 Risk estimate for the detection of *Listeria monocytogenes* genotypes in bulk tank milk samples when detected in other sampling sites

Sampling site	Fisher's Exact Test	RR [CI 95%] ^a
Milking system ^b	p<0.000	7.29 [4.48–11.85]
Udders & udder wipes	NS ^c	2.58 [1.11–5.98]
Feed surfaces	NS	2.01 [0.87–4.63]
Milking station floor	NS	1.96 [0.88–4.37]
Holding pen floor	NS	1.78 [0.69–4.58]
Bedding	NS	1.77 [0.69–4.58]
Feed	NS	1.77 [0.73–4.29]
Water troughs	NS	1.43 [0.62–3.29]
Milk room floor	NS	1.41 [0.53–3.74]
Feces	NS	0.83 [0.30–2.28]

^aRR [CI 95%]: relative risk and the 95% confidence interval

^bMilking system: milk filter tube, milk collector, milk tank outlet, teat cups and rinse water from the milking line

^cNS: not significant at the 95% confidence level

TABLE S5 Risk estimate for the detection of *Listeria monocytogenes* genotypes in milk filter socks by genotypes when detected in other sampling sites

Sampling site	Fisher's Exact Test	RR [CI 95%] ^a
Udders & udder wipes	p=0.003	2.41 [1.59–3.67]
Milking system ^b	NS ^c	2.27 [1.36–3.78]
Bedding	NS	1.41 [0.78–2.55]
Milk room floor	NS	1.41 [0.53–3.74]
Feed surfaces	NS	1.26 [0.72–2.21]
Holding pen floor	NS	1.12 [0.60–3.48]
Milking station floor	NS	1.07 [0.51–1.59]
Feed	NS	0.97 [0.65–1.99]
Water troughs	NS	0.93 [0.53–1.62]
Feces	NS	0.45 [0.20–1.86]

^aRR [CI 95%]: relative risk and the 95% confidence interval

^bMilking system: includes milk filter tube, milk collector, milk tank outlet, teat cups and rinse water from the milking line

^cNS: not significant at the 95% confidence level

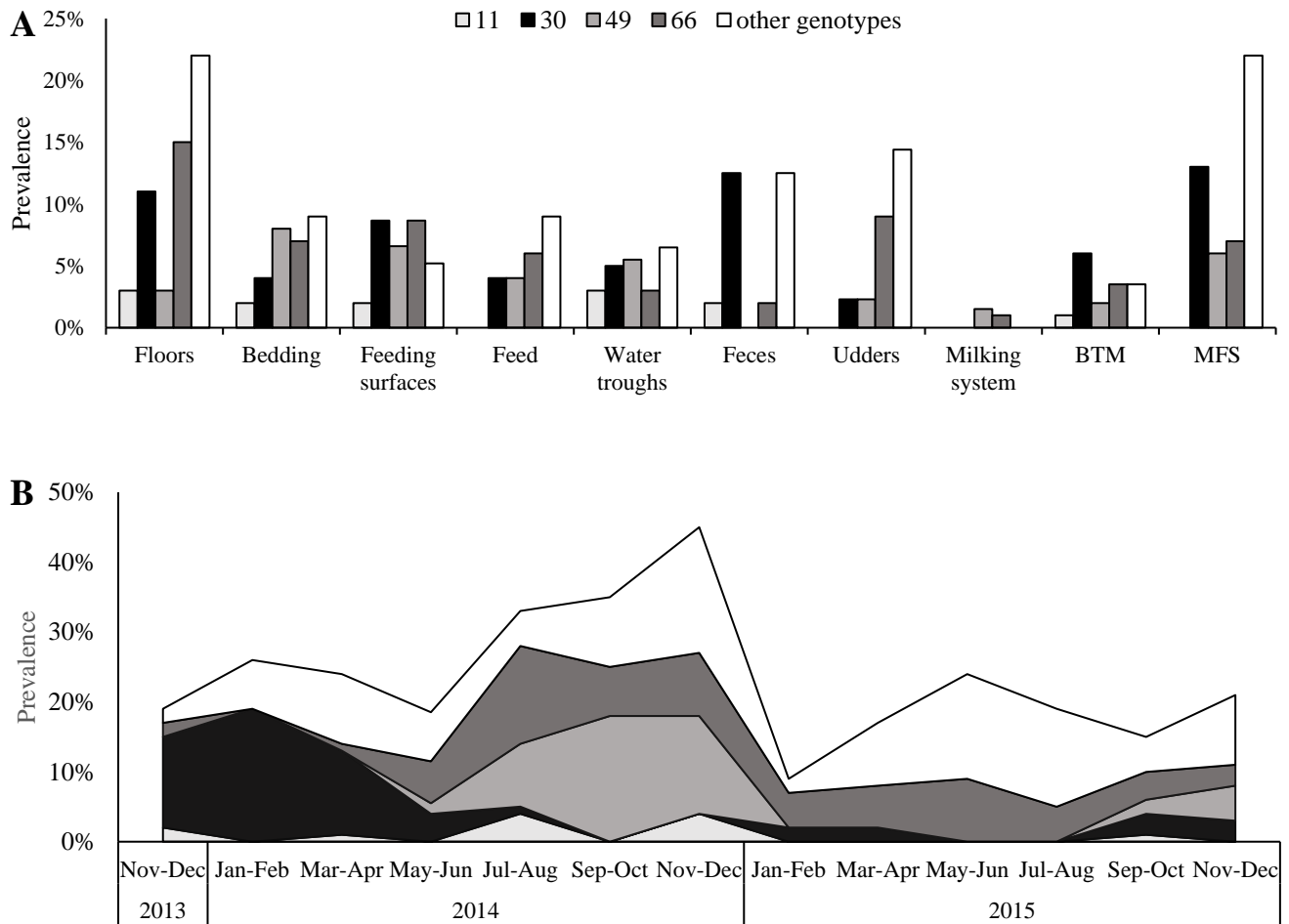


FIG. S1 Prevalence of persistent predominant genotypes (11, 30, 49, 66) and other *L. monocytogenes* genotypes on farm A by sampling site (A) and season (B). Samples of bulk tank milk (BTM) and milk filter socks (MFS) were collected in 75 samplings conducted every 1–2 weeks, and samples of the farm environment were collected in 13 bimonthly samplings, from November 2013 to November 2015.

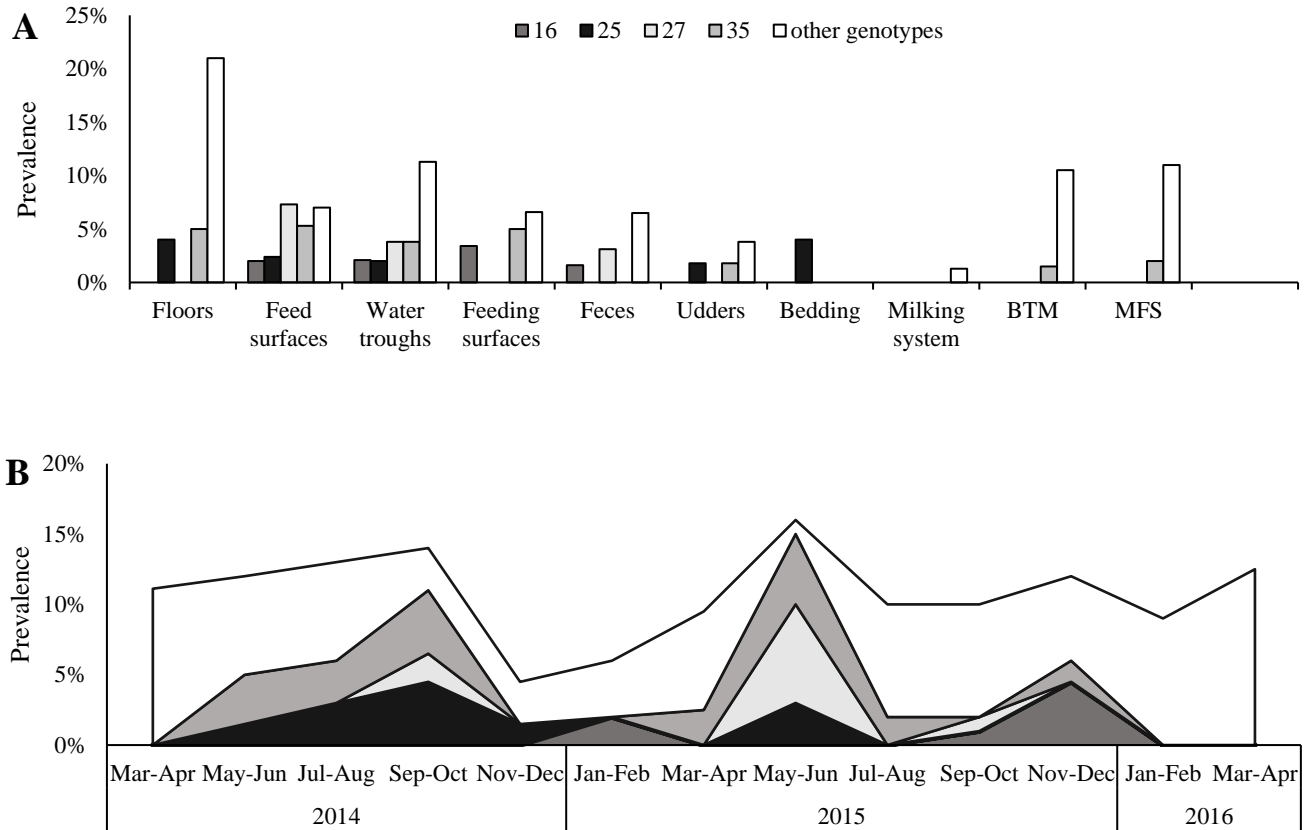


FIG. S2 Prevalence of persistent predominant genotypes (16, 25, 27, 35) and other *L. monocytogenes* genotypes on farm B by sampling site (A) and season (B). Samples of bulk tank milk (BTM) and milk filter socks (MFS) were collected in 59 biweekly samplings, and samples of the farm environment were collected in 13 bimonthly samplings, from April 2014 to April 2016.

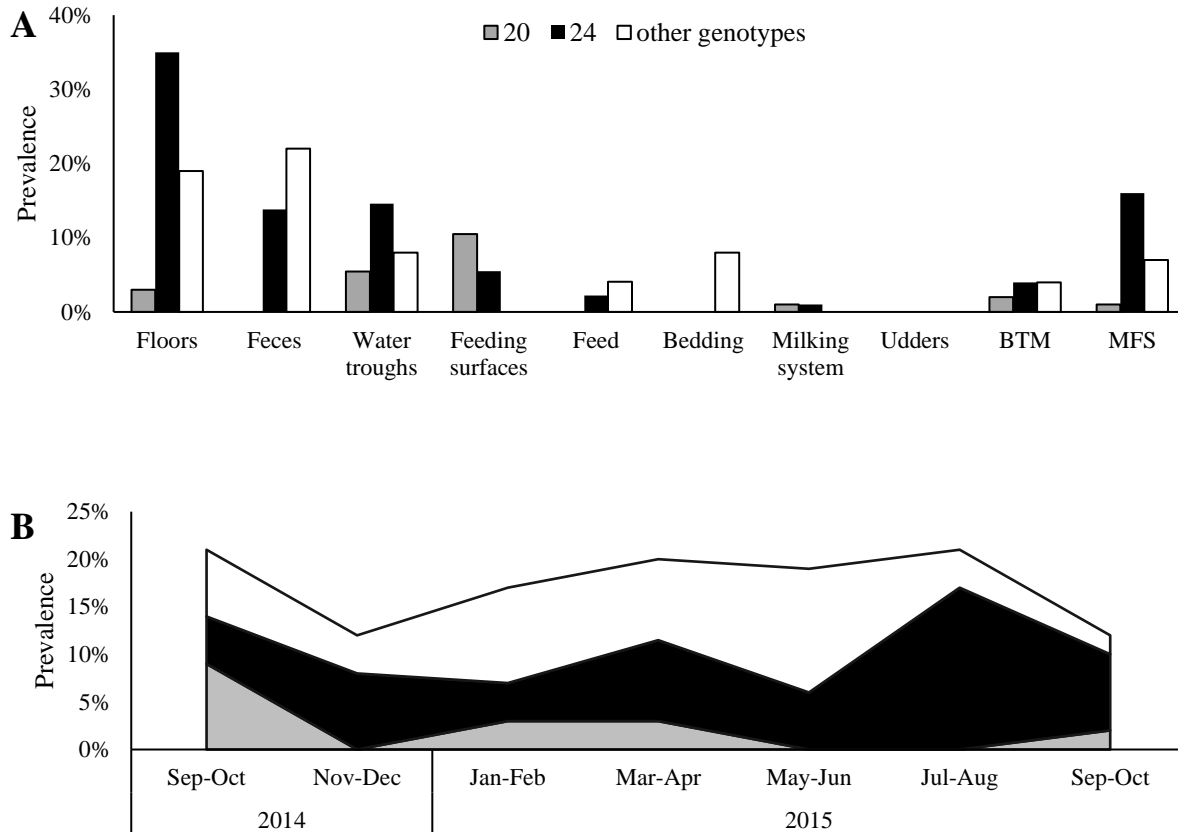


FIG. S3 Prevalence of persistent predominant genotypes (20, 24) and other *L. monocytogenes* genotypes on farm C by sampling site (A) and season (B). Samples of bulk tank milk (BTM) and milk filter socks (MFS) were collected in 54 weekly samplings, and samples of the farm environment were collected in 7 bimonthly samplings, from September 2014 to September 2015.

	BTM & MFS	Milking system	Udders	Bedding	Feeding surfaces	Feed	Waiting area floor	Milk room floor	Water troughs	Milking station floor	Feces
BTM & MFS		p<0.05	p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
Milking system	p<0.05		NS	NS	p<0.01	NS	NS	NS	p<0.01	p<0.05	NS
Udders	p<0.05	NS		p<0.01	NS	p<0.05	p<0.05	NS	p<0.05	NS	NS
Bedding	NS	NS	p<0.01		p<0.01	p<0.05	p<0.05	p<0.01	p<0.01	NS	p<0.05
Feeding surfaces	NS	p<0.01	NS	p<0.01		p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
Feed	NS	NS	p<0.05	p<0.05	p<0.01		NS	NS	p<0.05	NS	NS
Waiting area floor	NS	NS	p<0.05	p<0.05	p<0.01	NS		p<0.01	p<0.01	NS	NS
Milk room floor	NS	NS	NS	p<0.01	p<0.01	NS	p<0.01		p<0.05	NS	NS
Water troughs	NS	p<0.01	p<0.05	p<0.01	p<0.01	p<0.05	p<0.01	p<0.05		p<0.05	p<0.05
Milking station floor	NS	p<0.05	NS	NS	p<0.01	NS	NS	NS	p<0.05		p<0.05
Feces	NS	NS	NS	p<0.05	p<0.01	NS	NS	NS	p<0.05	p<0.05	

FIG. S4 Heat map of Fisher’s exact test results describing associations in the prevalence of *L. monocytogenes* genotypes between different sampling sites in the farm environment, bulk tank milk (BTM) and milk filter socks (MFS). Dark gray cells marked with “NS” indicate that the association was not statistically significant ($p \geq 0.05$), gray cells marked with “ $p < 0.05$ ” indicate that the association was significant ($0.01 \leq p < 0.05$), and light gray cells marked with “ $p < 0.01$ ” indicate that the association was highly significant ($p < 0.01$). Water through samples include both surface swab and water samples. Feeding surfaces include feed troughs (farms A–C) and feed tables (farms A and B). Udder samples include surface swab samples of uncleaned udders and used udder wipes, and samples of the milking system include surface swab samples of the milk filter tube, milk collector, milk tank outlet, teat cups and rinse water from the milking line.

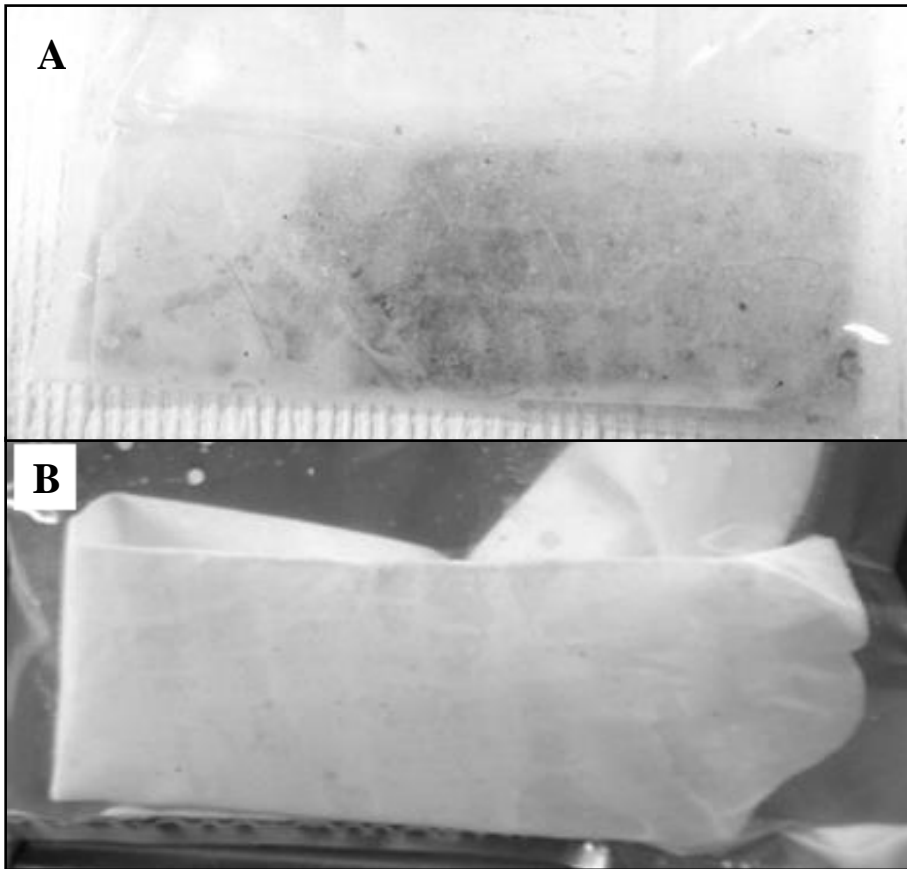


FIG. S5 Visual inspection of the in-line milk filter socks. A: Milk filter sock with copious visible debris, obtained from farm A. B: Milk filter sock with little visible debris, obtained from farm B.

Appendix S1.

Survey of deviations in farm routines prior to bulk tank milk and filter sock sampling

Instructions: Fill this survey at each milk and filter sock sampling to report any deviations from normal farm routines during the seven days preceding the date of sampling.

Name of the farm:

Date:

1. Were any abnormalities in animal health observed during the past week? Yes / No
 - If yes, what?
2. Were there any changes to farm staff or working routines during the past week (employment of new staff, substitute workers, etc.)? Yes / No
 - If yes, what?
3. Were any new animals purchased to the farm during the past week? Yes / No
 - If yes, what animals and how many?
4. Were any non-routine cleaning procedures or maintenance operations performed at the farm during the last week? Yes / No
 - If yes, what?
5. Were there any changes in feeding or feed quality during the past week? Yes / No
 - If yes, what?
6. Were there any visitors to the farm during the past week? Yes / No
 - If yes, describe the purpose of the visit:
7. Observations regarding sampling:
8. Additional comments: