

Febrile gastroenteritis after eating on-farm manufactured fresh cheese – an outbreak of listeriosis?

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SUMMARY

An outbreak of febrile gastroenteritis affected consumers of on-farm manufactured dairy products from a summer farm in Sweden. Symptoms included diarrhoea, fever, stomach cramps and vomiting in 88, 60, 54 and 21% of cases identified. The median incubation period was 31 h. A cohort study with 33 consumers showed an attack rate of 52% and an association between the total amount of product eaten and illness ($P=0.07$). Twenty-seven of 32 (84%) stool samples cultured for *Listeria monocytogenes* tested positive, although there was no association between clinical disease and the isolation of *L. monocytogenes*. In addition, gene sequences for VTEC and ETEC were detected in 6 and 1 subjects, respectively. Bacteriological analysis of cheese samples revealed heavy contamination with *L. monocytogenes* and coagulase positive staphylococci in all of them and gene markers for VTEC in one of them. Molecular profiles for *L. monocytogenes* isolated from dairy products, stool samples and an abscess from 1 patient who developed septic arthritis were identical. Results of both microbiological and epidemiological analyses point to *L. monocytogenes* as the most likely cause of this outbreak. The finding of markers for VTEC in some humans and cheese samples means that a mixed aetiology at least in some cases cannot be conclusively ruled out.

INTRODUCTION

The outbreak

At the end of June 2001, general practitioners (GPs) in Gävleborg County were alerted by an increase in patients with gastroenteritis who had been visiting a

local summer farm. This farm provided on-farm manufactured fresh cheese made out of cow's and/or goat's milk as well as butter. Visitors to the summer farm were offered tastings of these products. Some visitors additionally purchased cheese and/or butter, which were consumed over the following days (up to a week).

The high number of patients detected by the GPs reporting a history of consumption of dairy products from the summer farm was deemed sufficient by the

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Environmental Health Authority to identify the consumption of these products as the source of the outbreak. Environmental and health inspectors and the Gaevleborg Veterinary Officer initiated a bacteriological investigation which revealed contamination with a large number of *Listeria monocytogenes* in on-farm manufactured cheese samples.

Epidemiological investigations were undertaken in order first to describe the clinical manifestations according to age, sex and medical underlying condition; second to investigate what types and amounts of on-farm manufactured dairy products were associated with illness. Further microbiological studies were performed in order to establish the aetiology of the outbreak. As *L. monocytogenes* was the most consistent microbiological finding in the patients and in the cheese samples, the association between clinical disease and the isolation of this organism from stool samples was investigated.

The hypotheses tested were:

- (1) The isolation of *L. monocytogenes* from stool samples was associated with disease.
- (2) Individuals with underlying conditions were more likely to develop illness.
- (3) There was a dose–response between the amount of cheese ingested and risk of illness.

MATERIALS AND METHODS

Epidemiological investigation

A case was defined as an individual who had consumed any on-farm manufactured dairy product from the farm between 15 June and 9 July 2001 and who developed two or more of the following symptoms: fever, diarrhoea (three or more loose stools in 24 h), vomiting, arthralgia, headache or body pain within 2 weeks after the consumption of these products. A non-case was an individual meeting all but the clinical criteria.

The Gaevleborg County Medical Office for Communicable Disease Control and the County Veterinary Office actively searched for people who had visited and consumed dairy products from the summer farm. Two groups were identified: a group of tourists from a bus tour, who had visited the farm on 3 July, and members of a local historical society, who had visited the farm on 7 July. Local GPs in Gaevleborg carried out active surveillance in order to detect cases attending regional clinics. Cases detected in this way were

asked to identify relatives or friends who had consumed on-farm manufactured dairy products from the summer farm.

A standardized questionnaire was sent to members of the bus and historical society groups, as well as cases and non-cases detected in Gaevleborg within 5 weeks after the onset of disease of cases. The questionnaire gathered information about age, sex, underlying medical conditions, consumption history and development of symptoms. The consumption history was recorded as:

- type of dairy product;
- total number of doses of dairy product eaten, with each dose being equivalent to 1 spoonful (approximately 15 cc);
- average number of doses of dairy product eaten per day.

The study thus included subjects from the following groups:

- composite cohort group
 - the bus tour group (19 persons);
 - the historical society group (23 persons);
- the Gaevleborg group. Cases detected by the Gaevleborg GPs and relatives who met the case definition (total 50 persons) plus non-cases named by these cases.

The composite cohort was used to address hypotheses 1 and 2 above. All cases from the three groups are included in the description of clinical features. Cases and non-cases from the three groups who had a stool sample analysed were also included in the analysis to address hypothesis 3.

Statistical analyses were carried out using EPI-Info 6.02 (CDC, Atlanta, USA/WHO, Geneva, Switzerland). Risk ratios (RRs) and odds ratios (ORs) were computed with 95% confidence intervals (CI). Logistic regression was carried out using JMP 4.0.2, SAS, USA. All reported *P*-values are two-sided.

Microbiological investigation of human subjects

Human stool specimens were cultured for salmonella, shigella, *Yersinia enterocolitica*, *Campylobacter* spp. and *L. monocytogenes*. Enteropathogenic *Escherichia coli* (VTEC, EPEC, ETEC and EIEC) strains were investigated by PCR [1, 2]. Viral enteropathogens were investigated by electron microscopy. RT-PCR was used for the investigation of Norwalk-like virus in the samples [3].

Microbiological investigation of on-farm manufactured dairy products

Cheese and butter samples from the farm as well as those provided by consumers were cultured for salmonella, *Campylobacter* spp., coagulase-positive staphylococci, *Clostridium perfringens*, *Bacillus cereus*, *L. monocytogenes* and presumptive *E. coli*. In addition, cheese samples were tested for enteropathogenic *E. coli* strains by PCR. *L. monocytogenes* was detected using an enrichment method [4]. For quantification of *L. monocytogenes* 4 × 0.25 ml of the primary enrichment broth (LBI) were spread onto four Oxford agar plates, i.e. 1.0 ml in total. When necessary, tenfold serial dilutions of 1 ml of LBI were made in peptone water. The dilutions were then surface-plated in 0.1 ml portions onto Oxford agar plates. The plates were incubated for 48 h at 37 °C. Presumptive colonies were counted and five from each positive sample were verified.

Typing of *L. monocytogenes* strains

Isolates of *L. monocytogenes* from human stool samples and dairy products were serotyped and further characterized by Restriction Enzyme Analysis (REA) followed by Pulsed-Field Gel Electrophoresis (PFGE) using the Gene Navigator system (Pharmacia, Sweden). The enzymes used for restriction were *AscI* and *ApaI*.

RESULTS

Survey response rate

The response rate of the survey was 88% (37/42) for the composite cohort group. Thirty-three members of this group had eaten some kind of on-farm manufactured dairy product from the referred farm and thus were included in the study. In Gävleborg, of the 50 cases identified by GPs, 28 (55%) returned completed questionnaires. Three further cases as well as 11 non-cases identified by these cases submitted also completed questionnaires. A total of 42 people from this group were included in the study.

Clinical picture

Symptoms of cases are presented in Table 1. Forty-eight people met the case definition: 17 from the composite cohort and 31 from the Gävleborg group. Of these 48 cases, 25 were female and 23 male. Their average age was 52 years (range 2–85). The date of

Table 1. Symptoms of cases (n=48), summer farm outbreak, Gävleborg County, Sweden, June–July 2001

Symptom	No. cases with symptom (%) n=48	Average duration in days (range)
Diarrhoea	42 (88)	4.3 (1–9)
Fever	29 (60)	3 (1–6)
Stomach cramps	26 (54)	4.2 (1–15)
Chills	22 (46)	2.4 (1–5)
Vomiting	10 (21)	ND*
Headache	7 (15)	1.5 (1–2)
Muscle, joint or generalized pain	14 (29)	ND
Nausea	2 (4.2)	ND
Rash	1 (2.1)	ND

* ND, No data.

onset of clinical illness for cases is shown in Figure 1 and their median incubation period for illness was 31 h (range 10–240).

Forty-three of 48 (89.6%) cases had gastrointestinal symptoms (either diarrhoea or vomiting). Twenty-seven of 42 patients with diarrhoea also had fever (Table 1). There were 5 patients with neither diarrhoea nor vomiting, but muscle/body pain and headache (all) and fever (2). The study population contained 3 pregnant women (2 in the composite cohort). Only 1 developed symptoms (vomiting, headache and tiredness). All 3 women were treated with amoxicillin and gave birth normally.

Only one person was hospitalized, a 64-year-old woman from the Gävleborg group with chronic arthritis on immunosuppressive therapy with corticosteroids, who developed diarrhoea as well as a septic *L. monocytogenes* joint abscess. To our knowledge, none of the other patients developed any complications or sequelae.

Risk factors for disease

Of the 33 subjects in the composite cohort, 17 fulfilled the case definition (Attack rate, AR=51.5%). ARs increased with both the total amount of cheese consumed and the average amount eaten per day (Table 2). Being aged between 46 and 60 years was associated with illness (RR=2.57). Five (15.1%) of the 33 subjects had an underlying medical condition: diabetes (2), pregnancy (2) and asthma (1) and 4 of them met the case definition (AR=80%).

Eating unknown type of cheese and cow's milk cheese was associated with becoming a case. However

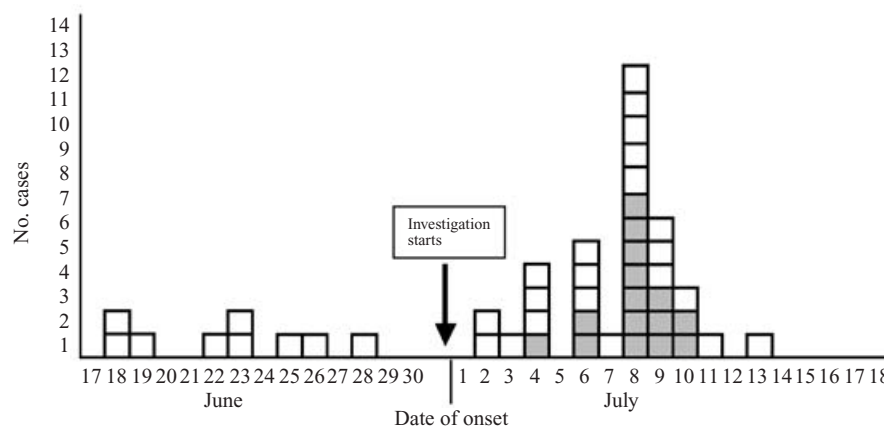


Fig. 1. Date of onset among cases. Summer farm outbreak, Gävleborg County, Sweden, June–July 2001. Shaded squares denote cases from the composite cohort, white squares those detected by the local GPs. Only cases who remembered date of onset of disease are shown ($n=45$).

Table 2. Attack rates for composite cohort, summer farm outbreak, Gävleborg County, Sweden, June–July 2001 ($n=33$)

	Cases	Total	AR (%)	RR	95% CI
Sex					
Males	6	12	50	Ref.	
Females	11	21	52	1.05	0.52–2.10
Age					
16–45	3	6	50	1.50	0.44–5.09
46–60	6	7	85.7	2.57	0.97–6.80*
61–75	5	11	45.4	1.36	0.44–4.21
76–99	3	9	33.3	Ref.	—
Underlying medical condition					
No	13	28	46.4	Ref.	—
Yes	4	5	80	1.72	0.95–3.11
Total no. doses†‡					
1–2	4	11	36.3	Ref.	
3–6	6	13	46.1	1.27	0.48–3.37
Over 6	7	9	77.8	2.14	0.91–5.04
Average no. doses per day§					
1–1.2	1	7	14.3	Ref.	
1.3–4	16	25	64.0	4.48	0.71–28.16

* Fisher exact test, $P=0.06$; † One dose of dairy product = 15 cc (=1 tablespoon); ‡ χ^2 for trend = 3.39 ($P=0.06$); § $P=0.032$.

no single type of cheese could explain all of the cases (Table 3).

The variables ‘being between 46 and 60 years of age’, ‘total no. of doses consumed’, and ‘average no. of doses consumed per day’ were included in a multivariate model to assess confounding and interaction. In the final model, only the total no. of doses consumed, not the other two variables, was significant (OR = 14.3; 95% CI = 0.93–341; $P=0.07$). The model

allowed the theoretical estimation of the infective dose 50% (ID₅₀%) in 5.1 doses (= 76 cc) of dairy product.

Thirteen of 17 cases of the cohort group (76.5%) had gastroenteritis. Of 6 who ate in total 2–4 doses, 3 (50%) developed illness compared to 10 (91%) of 11 who ate 5–11 doses ($P=0.09$).

Microbiological investigation of human subjects

Stool samples from the composite group (2 cases and 1 non-case) and the Gävleborg group (10 cases, 2 non-cases) which were cultured for common bacterial gastrointestinal pathogens, tested negative. Ten of these samples analysed for viral enteropathogens tested negative.

Samples from 43 individuals belonging to the composite cohort group (9 cases and 2 non-cases) and the Gävleborg group (23 cases and 9 non-cases) were cultured for *L. monocytogenes*. All (11/11) stool samples obtained from members of the composite cohort and 25/32 (78.1%) samples from the Gävleborg group yielded *L. monocytogenes*. Twenty-seven of 32 cases (84%) sampled yielded a *L. monocytogenes* positive culture, compared to 9 of 11 non-cases (82%). The culture from the abscess of the case with chronic arthritis also yielded *L. monocytogenes*.

Stool samples from 35 subjects belonging to the composite group (7 cases, 2 non-cases) and the Gävleborg group (19 cases, 7 non-cases) were tested for enteropathogenic *E. coli* strains. In the stool samples from 6 individuals, the gene markers for VTEC were found. All 6 had the gene sequence for *vt2* and 5 of them had also the gene sequence for *eaeA*. Additionally, in the stool sample from an individual, the genetic marker for ETEC (*eltB*) was detected. All

Table 3. Cheese-specific attack rates for composite cohort of on-farm manufactured dairy product consumers. Summer farm outbreak Gaevleborg County, Sweden, June–July 2001 ($n=33$)

Type of cheese	Exposed			Not exposed			Relative risk (95% CI)	% cases exposed
	Ill	Total	AR (%)	Ill	Total	AR (%)		
Blended milk	8	12	66.7	9	21	42.8	1.56 (0.82–2.94)	47.0
Cow's milk	4	4	100	13	29	44.8	2.23*(1.49–3.34)	23.5
Whey cheese	6	11	54.5	11	22	50	1.09 (0.55–2.16)	35.3
Goat's milk	9	21	42.8	8	12	66.7	0.64 (0.34–1.21)	52.9
Unknown type	4	4	100	13	29	44.8	2.23*(1.49–3.34)	23.5

* Significant 95% CI.

7 individuals with markers for enteropathogenic *E. coli* were also positive for *L. monocytogenes*. Two of the VTEC-positive individuals (*vt2* and *eaeA*) were non-cases.

Microbiological investigation of on-farm manufactured dairy products

Thirteen cheese samples (5 from goat's milk, 2 from cow's milk, 5 from blended milk and 1 whey cheese sample) and 1 butter sample were analysed. All samples were positive for the presence of *L. monocytogenes* and all but the whey cheese sample (not analysed) and the butter sample were positive for coagulase-positive staphylococci. Contamination levels in the analysed cheese samples ranged from 3.0×10^1 to 6.3×10^7 c.f.u. g^{-1} (*L. monocytogenes*) and 1.2×10^4 to 1.2×10^7 c.f.u. g^{-1} (coagulase-positive staphylococci). Goat's and blended milk cheese samples harboured a median of 4.4×10^6 and 3.1×10^6 c.f.u. g^{-1} of *L. monocytogenes* and 9.1×10^4 and 4.5×10^4 c.f.u. g^{-1} of coagulase-positive staphylococci, respectively. The cow's milk cheese samples contained an average 5×10^3 c.f.u. g^{-1} of *L. monocytogenes* and 3×10^6 c.f.u. g^{-1} of coagulase-positive staphylococci. The whey cheese and the butter samples contained 5×10^3 and 3×10^1 c.f.u. *L. monocytogenes* g^{-1} , respectively. Other bacteria found in the cheese samples included presumptive *E. coli* in varying quantities (<100 – 1.6×10^6 c.f.u. g^{-1}). One of the 13 cheese samples analysed harboured the gene markers for *vt2* and *eaeA*.

Typing of *L. monocytogenes* strains from humans and dairy products

All *L. monocytogenes* isolates obtained from dairy products (14) and stool samples (36) as well as the

abscess (1) belonged to serogroup 1/2a and showed identical restriction enzyme profiles.

Significance of *L. monocytogenes* isolation from human stool samples

The average number of days between last consumption of dairy product and sampling was similar for cases and non-cases (9.7 and 7.9 days, respectively) ($P=0.97$). Univariate analysis did not show any association between clinical disease and the isolation of *L. monocytogenes* from the stool samples. Nor we found any significant differences in age or gender between those with a positive culture and those with not (data not shown).

The analysis however showed a positive association between increasing amounts of dairy product consumed and the isolation of *L. monocytogenes*, and a negative association between length of time between last consumption to sampling and the isolation of the organism (Table 4).

A logistic regression model fitted with these two variables ('total no. of doses of dairy product' and 'interval last consumption to sampling') showed that both were independently associated with the isolation of *L. monocytogenes* ($P=0.06$ and $P=0.04$, respectively).

DISCUSSION

This study documents an outbreak of illness mainly characterized by gastroenteritis and fever following the ingestion of dairy products heavily contaminated with *L. monocytogenes*. The high contamination of the cheese with *L. monocytogenes*, the identical profiles of human and cheese isolates, the clinical features of the cases and finally, the evidence of invasive listeriosis in one of the patients, are factors that incriminated

Table 4. *Univariate analysis of factors related to the isolation of L. monocytogenes in stool samples from 32 cases and 11 non-cases. Summer farm outbreak, Gaevleborg County, Sweden, June–July 2001*

	Total	<i>L. monocytogenes</i> positive (%)	OR	95% CI
Clinical illness*				
Yes	32	27 (84.3)	1.20	0.13–9.23
No	11	9 (81.8)	Ref.	—
Diarrhoea				
Yes	31	26 (83.8)	1.04	0.12–7.85
No	12	10 (83.3)	Ref.	—
Total no. doses†‡				
1	6	2 (33.3)	Ref.	—
2–5	23	20 (86.9)	13.3	1.21–200.9
Over 5	14	14 (100.0)	25	1.60–857
Interval from last consumption to sampling (days)§				
1–7	18	18	13.5	0.83–456.41
8–14	17	13 (76.5)	2.44	0.27–23.78
Over 14	7	4 (57.1)	Ref.	—

* According to the case definition. † One dose of dairy product = 15 cc (= 1 tablespoon); χ^2 for trend calculations: ‡ 6.55; § 4.88; both $P < 0.05$; One (1) added to cell for calculation of OR.

L. monocytogenes in the aetiology of the outbreak. Moreover, soft cheese has often been incriminated in outbreaks of listeriosis [5–9]. Previous reports have shown that *L. monocytogenes* may cause a gastrointestinal illness with fever without progression to invasive illness in previously healthy individuals [10–14].

Febrile gastroenteritis involving *L. monocytogenes* has not previously been reported in Sweden. The clinical features of this outbreak were consistent with gastrointestinal listeriosis. However the proportion of cases reporting headache was lower (15%) compared to other studies published (65–88%) [10, 12, 14]. A plausible explanation for this discrepancy may be that headache is one of the easiest symptoms to forget, especially given that some time elapsed between the disease and the completion of the questionnaires. The incubation time (31 h) was similar to the 20–28 h described by other investigators [12–14].

We did not find any difference in susceptibility to illness by age or sex. However the risk for consumers with an underlying condition was short of statistical significance, which was probably due to the small study population.

Some of the investigated samples of cheese from the summer farm harboured over 10 million c.f.u. *L. monocytogenes* g⁻¹. These results may however,

overestimate the contamination of the ingested product, given that some of the samples were stored for 1–2 weeks in the consumer's refrigerator. It is well known that the bacterium can grow well at low temperatures [15].

It may be argued that *L. monocytogenes* was not the cause of the outbreak as there was no association between clinical illness and the isolation of the organism from stool samples. However, the high carriage frequency (81.8%) in non-cases who also ate the cheese may not be unexpected given that all cheese samples investigated harboured large numbers of *L. monocytogenes*.

Our findings contrast with those of Dalton et al. (1997) [12]. In an outbreak of febrile gastrointestinal listeriosis following the ingestion of contaminated milk, they reported a 37% isolation frequency for cases and zero for the six non-cases investigated who drank the milk. This discrepancy may be explained by a higher infective dose of the products consumed in our study. Unfortunately, we did not gather information about food preferences and previous exposure to similar kinds of food products among the study participants in order to understand this high frequency of carriage. It may be that in many individuals the organism was being temporarily excreted after the ingestion of the contaminated product. However, it is worthwhile emphasizing that in a considerable number of individuals the organism could be isolated from stool samples delivered up to 19 days after their last ingestion of dairy product. This presupposes that multiplication of the bacteria must have occurred in these individuals, supporting the hypothesis that the organism can be a commensal, causing sub-clinical infection. Müller [16] studied the frequency of isolation of *L. monocytogenes* in patients with diarrhoea and healthy food handlers and found a similar, but very low (less than 1%) frequency of carriage in both populations. Other investigators also have reported asymptomatic gastrointestinal carriage of *L. monocytogenes* bacteria in household contacts of *L. monocytogenes* patients [17].

On the other hand, when investigating a food-borne outbreak the agent will be found in some non-cases, since for almost all food-borne infections there will be persons excreting the pathogen agent without being ill. This would be particularly evident if the strain of organism in question is not highly virulent. Likewise, a pathogen will rarely be found in 100% of the diseased persons.

The mechanism of action of *L. monocytogenes* in the intestine is largely unknown. Although no enterotoxin

has to date been identified, it is possible that other exotoxins and extracellular enzymes can exert enterotoxin activity [18].

The role that micro-organisms other than *L. monocytogenes* may have played in the outbreak also merits discussion: the cheese samples also harboured large numbers of coagulase-positive staphylococci (1.2×10^4 to 1.2×10^7 c.f.u. g⁻¹) and some samples contained large numbers of presumptive *E. coli* (up to 1.6×10^6). In addition, gene sequences for *vt2* and *eaeA* (VTEC) were detected in one cheese sample. Previous studies have found that while both coagulase-positive staphylococci and presumptive *E. coli* are more or less part of the normal flora in Swedish raw milk cheese [19] *L. monocytogenes* is only detected in 0.5–1.0% of the samples analysed (unpublished data). As VTEC is considered as a part of the normal flora in ruminants [20], it is likely that a small part of the presumptive *E. coli* encountered in the cheese samples were VTEC. All VTEC encountered in ruminants are, however, not considered as potential EHEC as additional virulence factors than production of verotoxin are needed for pathogenicity [21]. The sparse findings of markers for VTEC in both human and cheese samples and the clinical picture do not clearly incriminate VTEC, although a mixed aetiology, at least in some cases, cannot be conclusively ruled out.

The clinical picture of the cases is not consistent either with *S. aureus* food poisoning, which presents with a much shorter incubation period (typically 1–6 h) and a very acute clinical presentation (less than 1–2 days) with frequent vomiting [22]. No patient in the present outbreak had an incubation time remotely close to what is expected in *S. aureus* poisoning.

Whereas no specific type of cheese alone could explain the outbreak the consumption of increasing amounts of cheese was associated with increased risk of disease. Given the high level of contamination of the dairy products with *L. monocytogenes*, this dose–response is not likely to reflect the chances of ingesting the organism, but implies that the total amount of ingested bacteria may play an important role in the induction of clinical disease. Nevertheless, 27 people ate highly contaminated dairy products and did not develop any clinical symptoms.

The incidence of this form of gastroenteritis in Sweden remains unknown, but it maybe more common than previously thought, given that cultures for *L. monocytogenes* are not routinely performed to investigate patients with gastroenteritis.

Furthermore, routine laboratory investigations do not find a causative agent in over 35% of samples of diarrhoea cases analysed [23].

Further investigations are needed in order to elucidate the role of *L. monocytogenes* in cheese-associated gastrointestinal disease. Cases of gastroenteritis among consumers and non-consumers of these types of dairy product should be investigated for *L. monocytogenes*. Further studies are also necessary in order to study the excretion of the organism and the risk of disease for contacts of cases.

The bacteriological quality of the dairy products suggest that a summer farm may not provide suitable hygienic conditions for making perishable foods such as raw milk fresh cheese. However and given the popularity of these enterprises in Sweden it is unlikely that this activity will cease. In that case, regular hygiene inspections of these farms and enforcement of strict hygienic measures should reduce contamination and thus keep the risk of disease to a minimum.

In summary, we investigated an outbreak using a combination of epidemiological and microbiological methods. Results of these investigations led to the closure of the summer farm to visitors by the environmental authorities. The most likely pathogen appeared to be *L. monocytogenes* which was demonstrated in the on-farm manufactured dairy products and the patients. The same evidence, to a lesser extent, was present for VTEC. We have to take into account that the mere storage of specimens and the time span between storage and culturing specimens are favourable factors for the isolation of *L. monocytogenes*. Nevertheless, we interpret these results in the direction of a possible major role for *L. monocytogenes* as a causative bacterial species behind this outbreak of gastrointestinal disease.

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