

Outbreak of *Yersinia pseudotuberculosis* O:1 infection associated with raw milk consumption, Finland, spring 2014

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In March 2014, a *Yersinia pseudotuberculosis* (YP) outbreak was detected by a municipal authority in southern Finland. We conducted epidemiological, microbiological and traceback investigations to identify the source. We defined a case as a person with YP infection notified to the National Infectious Disease Registry between February and April 2014, or their household member, with abdominal pain and fever $\geq 38^\circ\text{C}$ or erythema nodosum. Healthy household members were used as household-matched controls. We identified 43 cases and 50 controls. The illness was strongly associated with the consumption of raw milk from a single producer. The odds ratio of illness increased with the amount of raw milk consumed. Also previously healthy adults became infected by consuming raw milk. Identical YP strains were identified from cases' stool samples, raw milk sampled from a case's refrigerator and from the milk filter at the producer's farm. The producer fulfilled the legal requirements for raw milk production and voluntarily recalled the raw milk and stopped its production. We advised consumers to heat the raw milk to 72°C for 15 s. Current legislation for raw milk producers should be reviewed and public awareness of health risks linked to raw milk consumption should be increased.

Background

Yersinia pseudotuberculosis (YP) is a pathogen transmitted to humans via the faecal-oral route through consuming contaminated water or food, especially raw and undercooked products [1]. It causes yersiniosis, a gastrointestinal disease characterised by abdominal pain and fever, which can lead to post-illness complications such as erythema nodosum and reactive arthritis [2].

The incubation period of YP is three to seven days [2] but can be up to 18 days [3].

In Finland, YP infection is a notifiable disease. Outbreaks cause large variation in the annual YP incidence, for example 4.8 per 100,000 in 2006 and 0.7 per 100,000 in 2013 [4]. Most cases are sporadic, but 10 YP outbreaks were reported between 1997 and 2008 [3]. Identified sources of previous YP outbreaks include carrots [3], iceberg lettuce [5] and vegetable juice [6].

On 27 March 2014, one local public health authority in southern Finland contacted the National Institute for Health and Welfare (THL) to notify four cases of YP infection. This was followed by a notification to the National Registry for Food and Waterborne Outbreaks. We investigated the outbreak to identify its extent and source in order to apply appropriate control measures and to prevent further cases and future outbreaks.

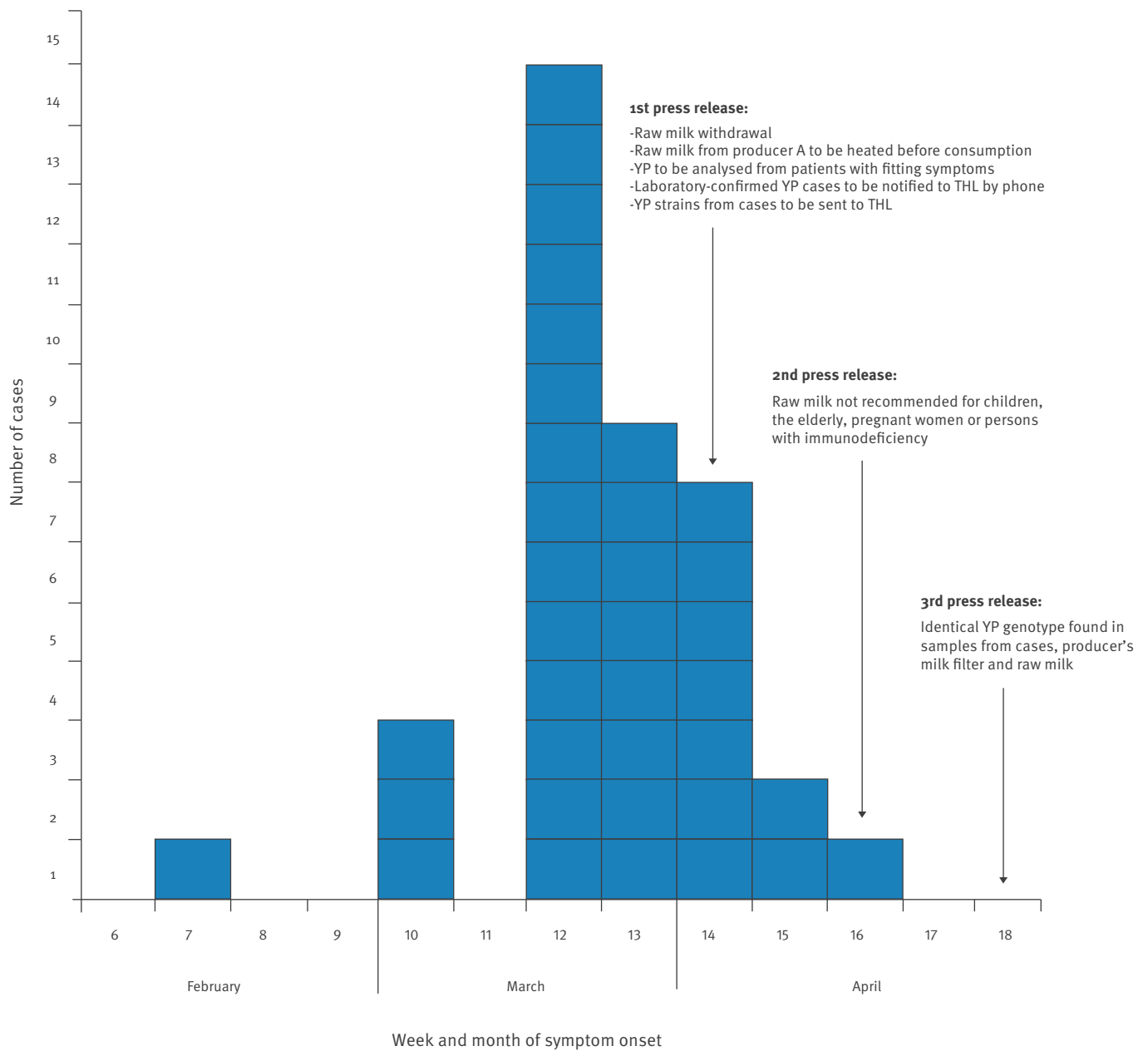
Methods

Case finding and hypothesis generation

In order to detect YP infection cases, we sent an alert to physicians and clinical laboratories all over Finland, asking them to take samples from patients with symptoms resembling yersiniosis and to report laboratory-confirmed cases to the outbreak investigation team by phone. Clinical laboratories are required by law to notify the National Infectious Disease Registry (NIDR) of any YP findings identified in stool or of antibodies in serum. We line-listed persons with YP infection notified to the NIDR from February to April 2014.

FIGURE 1

Number of *Yersinia pseudotuberculosis* cases by week of symptom onset and timeline of public health events, Finland, February–April 2014 (n = 36)



THL: National Institute for Health and Welfare; YP: *Yersinia pseudotuberculosis*.

Date of symptom onset available for 36 cases.

A public health nurse interviewed six persons from the line list, using a standard trawling questionnaire to generate hypotheses. The trawling questionnaire for gastrointestinal illnesses contained 97 questions on the clinical picture of the disease, travel history, consumed foods and drinks, cooking methods, sites for shopping and eating out, and animal contact.

Case-control study and statistical analysis

We conducted a case-control study to test the hypothesis suggested by the trawling interviews. Our study

population comprised persons with YP infection notified to the NIDR and their household members. The outbreak team at THL developed a web-based questionnaire. This included questions about the demographical and clinical characteristics of the subjects, onset of the symptoms, food exposure suggested by trawling interviews and by literature as previously identified sources of YP outbreaks, and about the frequency and amount of foods consumed during the two weeks before the onset of symptoms. Respondents

TABLE 1

Exposure to food items among cases and controls (matched univariate analysis), *Yersinia pseudotuberculosis* outbreak, Finland, February–April 2014 (n = 93)

Variable	Cases (n = 43)	Controls (n = 50)	mOR (95% CI)	p value
	Exposed n (%)	Exposed n (%)		
Raw milk (producer A)	42 (98)	24 (48)	22.2 ^a (3.6–∞)	<0.001
Raw milk (in general)	42 (98)	30 (60)	16.9 ^a (2.6–∞)	0.001
Salad	34 (79)	40 (80)	1.7 (0.1–117.8)	1.000
Raw milk (producer other than A)	1 (2)	2 (4)	1.5 ^a (0.0–58.5)	1.000
Other vegetable	26 (60)	38 (76)	0.9 ^a (0.1–∞)	1.000
Carrot	38 (88)	41 (82)	0.8 (0.0–49.1)	1.000
Vegetable juice	0 (0)	4 (8)	0.56 ^a (0.0–7.5)	0.667

CI: confidence interval; mOR: matched odds ratio.

^a Median unbiased estimate.

TABLE 2

Unadjusted and adjusted dose–response relationship between consumed raw milk from producer A and *Yersinia pseudotuberculosis* infection, Finland, February–April 2014 (n = 93)

	Cases (n = 43)	Controls (n = 50)	Total (n = 93)	Univariate		Multivariable ^a	
	n (%)	n (%)	n (%)	mOR (95% CI) ^b	p value	mOR (95% CI) ^b	p value
Quantity of consumed milk							
None	2 (8)	24 (92)	26 (100)	Reference		Reference	
<1 dL/day	9 (56)	7 (44)	16 (100)	7.2 (0.71–∞)	0.098	10.7 (0.99–∞)	0.051
1–3 dL/day	19 (63)	11 (37)	30 (100)	14.5 (2.21–∞)	0.003	8.0 (1.09–∞)	0.039
>3 dL/day	12 (71)	5 (29)	17 (100)	20.0 (2.07–∞)	0.008	12.2 (1.26–∞)	0.029
Age						0.92 (0.85–0.97)	<0.001

CI: confidence interval; mOR: matched OR.

^a Adjusted for age.

^b Median unbiased estimator of OR; univariate p value for trend is <0.001; multivariable p value for trend is 0.016.

TABLE 3

Environmental samples tested during a *Yersinia pseudotuberculosis* outbreak, Finland, February–April 2014 (n = 21)

	Sample (n positive/n tested)				
	Milk filter	Bovine faeces	Raw milk (bulk tank)	Raw milk (case's refrigerator)	Raw milk (producer's storage)
<i>Yersinia pseudotuberculosis</i>	2/3 ^a	2/9 ^a	5/5	1/1 ^a	3/3
YP PFGE outbreak pattern	1/1	0/2	0/0	1/1	0/0
YP MLVA outbreak profile	1/1	0/2	0/0	1/1	0/0
<i>Campylobacter jejuni</i>	2/3	9/9	0/5	0/1	0/3
Presumptive STEC ^b	3/3	0/0	4/5	0/0	0/0
<i>Yersinia enterocolitica</i>	1/3	0/0	0/5	0/1	1/3

MLVA: multilocus variable-number of tandem repeats analysis; PFGE: pulsed-field gel electrophoresis; STEC: *Shiga toxin-producing Escherichia coli*. YP: *Yersinia pseudotuberculosis*.

^a All YP serotype O:1.

^b Presumptive STEC is defined as real-time PCR detection of (i) the *stx* gene, (ii) *stx* and *eae* genes or (iii) *stx* and *eae* genes as well as genes associated with one of the serogroups O157, O111, O26, O103 or O145.

were invited to reply using either a web-based questionnaire or a paper form.

We defined a case as a person with YP infection notified to the NIDR or with symptoms of abdominal pain and fever of $\geq 38^{\circ}\text{C}$ or erythema nodosum in the period from February to April 2014. The matched controls were respondents living in the same household as the case who did not meet the case definition.

We calculated matched odds ratios to explore the associations between a single exposure and the outcome. To control for possible confounders, significant variables in the univariate analysis were included in the multivariable model. Households with only one member or no controls were excluded from the matched analysis. We coded the responses 'do not remember' or 'do not know' as missing values. We used exact conditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for both univariate and multivariable analyses. Where a finite conditional maximum likelihood estimate did not exist, the median unbiased estimator (MUE) of the OR was calculated [7].

To calculate the point estimates for the effects of exposure doses in the univariate analysis, we used binary variables for each milk dose in the model. In the multivariable analysis, we adjusted the model for continuous age. The significance of the dose-response trend was assessed using the ordinal level dose-response variable as continuous both in the univariate and in the multivariable analysis. For all analyses, a p value of < 0.05 was considered significant. The STATA Data Analysis and Statistical Software version 12 (Stata Corporation, College Station, United States) was used to perform the analysis.

Traceback and microbiological investigation

After raw milk was identified as a possible source of the outbreak, the municipal authority contacted the producer in order to investigate the production procedure, to identify when, where and how much of the specific product was distributed and to find out whether the producer followed the relevant regulations for food production.

On 4 April, an opened 3L package of raw milk from a specific producer (with 'use by' date on 1 April) was sampled from a case's refrigerator. On 7 April, the local team visited the producer's farm to collect the milk filter, bulk tank milk and bovine faecal samples. Bovine faecal samples were pooled to represent ca 10 animals each. Unopened 3L packages from the producer's storage were also collected.

Stool specimens from the patients were analysed at four clinical microbiology laboratories using faecal culture and a quantitative PCR for *Salmonella*, *Yersinia*, *Campylobacter*, *Vibrio cholera*, *Shigella*, and *Shigatoxin-producing Escherichia coli* (STEC) [8]. Milk, milk filter and bovine faecal samples were analysed

at the Finnish Food Safety Authority Evira. Screening of environmental samples for YP, pathogenic *Yersinia enterocolitica* and STEC was conducted by real-time PCR [9-11]. For *Y. enterocolitica* detection, we followed the technical specification ISO/WD 10273 [12] and for STEC detection we followed ISO/TS 13136 [11], where presumptive STEC is defined as real-time PCR detection of (i) the *stx* gene, (ii) *stx* and *eae* genes or (iii) *stx* and *eae* genes as well as genes associated with one of the serogroups O157, O111, O26, O103 or O145.

For isolation of YP, samples of 10 g (faeces) 25 mL (milk) or one half of a milk filter were homogenised in phosphate-mannitol-peptone (PMP) broth [13] as described previously [3]. The growth characteristics of YP in bulk tank milk were studied by plating dilutions of the refrigerated tank milk on CIN agar on day 0, 1, 2, 5, 7, 9 and 12 after collection (up to one day after milking). Each sample was plated in duplicate and the average count of YP colonies was used. YP isolates were identified by API 20E (bioMérieux, France) and MALDI-Biotyper (Bruker Daltonics, GmbH). Isolation of *Campylobacter* spp. from milk, milk filter and bovine faecal samples was carried out according to ISO 10272-1:2006 [14] with a modification in the enrichment: an incubation time of 24 hours without a pre-incubation step was used.

Serotyping, pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat analysis (MLVA) were performed at THL to characterise and compare the isolates [5,15]. No band differences were allowed in PFGE and a single-locus difference was allowed in MLVA to designate two strains as indistinguishable. *C. jejuni* isolates were subtyped at Evira with PFGE using *SmaI* and *KpnI* [16].

Results

Descriptive and analytical epidemiology

From February to April 2014, we identified and line-listed 55 persons with YP infection registered in the NIDR. They represented 48 households and their median age was 14 years (range: 1-67). Of those 55, 35 were men and 51 were from the Helsinki and Uusimaa hospital district in the south of Finland. In those three months, the incidence rate of YP infection cases in this hospital district was more than six times higher (incidence rate ratio (IRR): 6.4; 95% CI: 3.0-15.6) than in the same period in 2013 (13.2/100,000 vs 2.1/100,000). All six persons completing the trawling interview reported consuming raw milk from a single producer A.

In total, 93 persons from 30 households (household response rate: 63%) completed the questionnaire. The online questionnaire was used by 53 of 93. Of the respondents, 43 were identified as cases (33 based on NIDR notification, 10 based on symptoms) and 50 as controls. Respondents under the median age of 23 years were 12 times more likely to be a case than those over the age of 23 (OR=12.1; 95% CI: 2.8-111.0;

$p < 0.001$). Of the 43 cases, 22 were men, and the median age was 13 years (range: 1–67). One case had a campylobacter co-infection.

The first case fell ill on 10 February, and the number of cases peaked in March, week 12 (Figure). The median duration of the illness was 14 days (range: 4–27). Abdominal pain and fever were the most commonly reported symptoms (39 and 31 of the 43 cases, respectively), followed by nausea, diarrhoea and joint pain (20, 19 and 13 of the 43 cases, respectively). Three cases developed erythema nodosum, and four were hospitalised. No significant difference ($p = 1.000$) was found between cases and controls regarding previously diagnosed chronic diseases (7/43 vs 9/50, respectively).

Eight households, comprising in total 13 cases, were excluded from the matched analysis because they had only one household member or no controls. In the univariate analysis, raw milk was the only food item significantly associated with the illness (Table 1) and an increase of one year in age decreased the odds of illness by 6% (OR=0.94; 95% CI: 0.90–0.98; $p < 0.001$). Compared with those who did not drink raw milk from producer A, the age-adjusted odds of illness were higher than 35 for those who consumed it (MUE of OR=35.2; 95% CI: 4.07–∞; $p < 0.001$). Of the 93 questionnaire respondents, 89 provided information on the volume of consumed raw milk. The OR of illness increased with the volume of raw milk consumed ($p = 0.016$) (Table 2).

Traceback and microbiological investigation

The raw milk was produced on a farm that had 90 milking cows and two regular daily employees. The majority of the milk produced was delivered to a dairy for pasteurisation. In February 2014, the farm started operating a packaging facility for the raw milk once per week. The product was first distributed to retail stores on 10 February. From 10 February to 3 April 2014, 11,835 L of this raw milk were delivered to 24 shops in southern Finland. The raw milk was sold in 3 L packages, with a 'use by' date five days from the date of packing. In compliance with Finnish regulations, all packages had a warning label, stating that the milk may contain harmful microbes and should be heat-treated before serving to risk groups, i.e. children, pregnant women, the elderly or individuals with a severe primary disease [17].

In the microbiological investigation, all of the 41 YP patient isolates that had been sent to the THL reference laboratory from February to April had the serotype O:1. Isolates from eight cases were characterised by PFGE and all showed an identical profile (outbreak profile). Seven of eight isolates were also analysed with MLVA and had the indistinguishable outbreak profile 5–9–3–2–4–8–12. YP with the outbreak profile was also detected in a milk sample from the refrigerator of a case and from the milk filter (Table 3). The

concentration of YP in tank milk increased steadily during storage at 4 °C. It was 2 colony-forming units (cfu)/mL when collected from the tank and 5 cfu/mL, 26 cfu/mL and 120 cfu/mL after one, two and five days of storage, respectively. After 12 days of storage, the concentration was 3,500 cfu/mL.

C. jejuni was detected by culture in environmental samples, and pathogenic *Y. enterocolitica*, presumptive STEC O103, O145 and STEC O103 were detected by PCR (Table 3). *C. jejuni* was not isolated from tank milk. Attempts at isolation did not yield any *Y. enterocolitica* or STEC isolates. A *C. jejuni* isolate from one patient was available for comparison with isolates from the milk filters ($n = 2$) and bovine faeces ($n = 7$). Identical PFGE profiles were identified.

Public health measures

After producer A's raw milk was identified as the suspected source of the outbreak, the producer voluntarily recalled a total of 200 L of raw milk from the market on 3 April 2014. The farm discontinued the commercial production of raw milk. The local authority, THL and Evira announced the outbreak in the media in order to inform the public about the possible contamination of the raw milk from a particular producer and to advise to heat it to 75 °C for 15 s before consumption (Figure).

Discussion

We describe the outbreak of 55 cases of YP infection that occurred between February and April 2014 in southern Finland. The YP infection was associated with the consumption of raw milk from a single producer, with 98% of the cases consuming raw milk from this source. Microbiological evidence supported the epidemiological findings. An identical YP serotype and genotype were found in isolates from the patients, the milk filter from producer's farm and raw milk from a case's fridge.

Critical selection of controls is necessary to avoid introducing bias in the study. We used a method in which any healthy responding household member was suitable for being a matched control for a case. Choosing controls among household members may result in overmatching [18]. Despite the fact that household members may share similar dietary habits, we were able to demonstrate a strong association between raw milk consumption and illness.

An increase in outbreaks related to raw milk contaminated by a variety of pathogens has been observed [19]. Pasteurisation is a well-known and effective way to eliminate pathogens and bacteria from raw milk, decreasing the risk of disease transmission [20]. THL and the Finnish Food Safety Authority Evira recommend that children, the elderly, pregnant women and persons with a severe primary disease should heat-treat raw milk before consumption. In this study, age was associated with illness: those under 23 years of age were more likely to get ill. By contrast, previously

diagnosed chronic diseases did not affect the probability of YP infection. Our results are in line with findings from a study showing that healthy adults can become infected by consuming contaminated raw milk [21], and we suggest that also adults should heat raw milk before consuming it.

This outbreak was identified because a cluster of YP cases visited the same hospital. In Finland, *Yersinia* and *Campylobacter* infection notifications are not monitored in real time, and strains isolated from these cases do not need to be sent to the national reference laboratory for typing. Therefore, detection of geographically widespread *Yersinia* or *Campylobacter* outbreaks and routine comparison of YP isolates would be impossible. No YP profile identical to the current outbreak strain was identified among 61 Finnish YP strains tested in a previous study [15]. In this outbreak, identification of a cluster of cases allowed a timely response and prompt control measures. The producer voluntarily discontinued raw milk production and withdrew the product from the market immediately after the trawling interviews were completed. Simultaneously, the national authorities informed the public and health-care professionals about health risks linked to raw milk consumption. No new YP cases were detected after the recall of the raw milk.

Despite the early detection and rapid control measures, this outbreak was larger than previously reported outbreaks linked to raw milk consumption [22]. Moreover, there were probably more cases than recorded. Additional cases on the basis of symptoms were detected by us in one in four of the responding households. Persons with severe symptoms are more likely to seek medical care and thus more likely to be sampled. It has been estimated that only ca 20% of persons contracting gastroenteritis consult a physician [23].

In this outbreak, the milk was contaminated by several organisms. The most probable route for contamination was through bovine faeces during milking. YP with an identical profile was detected in patients and bovine faeces. In addition, an identical *C. jejuni* profile was identified in one patient, bovine faeces and the milk filter. Raw milk may also become contaminated via cleaning water, cow mastitis or cross-contamination through humans or rodents [24]. Rodents and other small mammals are the main reservoirs for YP [2]. In previous YP outbreaks in southern Finland, rodents on farms were suggested as a possible source of YP transmission [3,25]. Further studies are needed to clarify the transmission dynamics of YP on farms.

In this study, the case from whose refrigerator raw milk had been sampled reported consumption of less than 1 dL of milk three days after the packaging date, and the amount of YP in tank milk was estimated at 26–120 cfu/mL at the same time point. While the infectious dose of YP is believed to be 10^8 or more bacteria if

ingested orally [26], ingestion of fewer than 10^4 cfu of YP could have sufficed for infection in this outbreak. YP may reach infectious concentrations in products stored for at least several days at refrigerator temperature, since it can multiply at 4 °C [27].

YP is one of the pathogens included in the European Food Safety Authority's shortlist of microbiological hazards, indicating that it can be transmitted to humans through the milk [28]. It is, however, seldom studied and little information is available about its prevalence in unpasteurised milk [18,29,30]. No YP was found in raw cow milk samples from bulk tanks collected from 183 dairy farms in Finland in 2011 [31]. Raw cow milk producers in Finland are required to test their milk for *Listeria monocytogenes*, *Campylobacter* and STEC O26, O103, O111, O145 and O157 but not for YP [17]. In this outbreak, the producer's raw milk fulfilled the requirements of the law. Five persons who had consumed this producer's raw milk had fallen ill with campylobacteriosis in March 2014, before the YP outbreak notification. At that time, their illness could not be associated with the raw milk because *Campylobacter* had not been found in the producer's raw milk. The current testing regime for *Campylobacter* may be insufficient, because the concentration of the pathogen may be below the threshold of what is detectable in milk. In our investigations in April 2014, an identical *C. jejuni* genotype was detected in samples of one YP patient, bovine faeces and the producer's milk filter. Examination of milk filters instead of bulk tank milk samples seems to increase the probability of detection of pathogens. In the filter, the concentration of pathogens is higher than in bulk tank milk because large volumes of raw milk flow through it. The milk filter could therefore be used as the standard for testing.

Current control measures for raw milk production are not sufficient to prevent illness in those drinking this product. In this outbreak, contamination occurred although the raw milk met the microbiological criteria. The producer also fulfilled other legal requirements: raw milk was labelled with information on the possible microbial health hazards, risk groups and appropriate storage conditions in order to protect the consumer [17]. Before starting production, the producer is required to prove that the dairy cattle are free from *Salmonella* and STEC O157. Specific requirements are in place for the farm buildings and operations on the farm to avoid contamination of milk through contact with manure. The milk has to be cooled immediately after milking and stored at 6 °C or cooler [17]. The producer also met these requirements. It is relevant to review the legislation and to increase the awareness of the general public and policymakers concerning the potential hazards of raw milk consumption.

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Conflict of interest

None declared.

Authors' contributions

TP contributed to the collection and analysis of data and drafted the manuscript as the lead writer. SHa and MH were responsible for laboratory analysis of food and environmental samples and contributed to the comparison of human, food and environmental samples. SS was responsible for the microbiological investigation of the human samples and contributed to the comparison of human, food and environmental samples. AP contributed to the trace-back investigation. SHe was in charge of the local outbreak investigation. HTN contributed to the local outbreak investigation and was in charge of patient management. JO contributed to the case-control study design and statistical analysis of data. SHU contributed to the questionnaire design and data collection. RRF was responsible for the epidemiological investigations and contributed to the case-control study design, questionnaire design and analysis of the data. SS, SHa, AP, MH, SHe, JO and RRF contributed to manuscript writing. All co-authors critically reviewed the draft of the paper and approved the final version.

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