
A community outbreak of *Salmonella berta* associated with a soft cheese product

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SUMMARY

In September 1994, a complaint was registered at a public health unit concerning a cheese product. In addition, public health laboratories in Ontario reported an increase in the number of isolates of *Salmonella berta* from patients with diarrhoeal illness. A clinical, environmental and laboratory investigation was initiated to determine the nature of this outbreak. Isolates of *Salmonella berta* were compared using large fragment genomic fingerprinting by pulsed-field gel electrophoresis (PFGE). By late October, 82 clinical cases had been identified including 35 confirmed, 44 suspected and 3 secondary. The investigation linked illness to consumption of an unpasteurized soft cheese product produced on a farm and sold at farmers' markets. Subtyping results of patient, cheese and chicken isolates were indistinguishable, suggesting that the cheese was contaminated by chicken carcasses during production. The outbreak illustrates the potential role of uninspected home-based food producers and of cross-contamination in the transmission of foodborne bacterial pathogens.

INTRODUCTION

Salmonella berta is a relatively uncommon cause of salmonellosis in Canada and, in 1993, this serotype accounted for only 74 of 8057 (0.9%) reported *Salmonella* serotypes isolated from humans [1]. On 26 September 1994, a complaint was registered at the Waterloo Regional Community Health Department (WRCHD), after the complainant and her aunt suffered diarrhoeal illness. The two women were ill on 19 September, 2 days after a common meal which included a cheese product purchased at a local

farmers' market. One of the women had sought medical attention and *Salmonella berta* was cultured from her stool. Given the source of the food, it was suspected to have been produced under unsanitary conditions in an unregulated home-based operation. The food sample provided by the complainant was cultured positive for *S. berta* and *Escherichia coli*. On 6 October, a public health inspector visited the farm where the cheese product was made. The same day, the Central Public Health Laboratory (CPHL), Ontario reported 26 *S. berta* isolations in the month of September, compared to an average of 8 per month in the previous 8 months. The WRCHU was informed that 12 of the 26 *S. berta* cases identified in September had occurred in their region, suggesting the possibility

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of a more widespread outbreak. These events prompted an investigation to confirm the existence of a community outbreak and determine the source.

METHODS

Case investigation

Cases were identified through laboratory reports and self-reporting by persons responding to a media release issued by the health department. A confirmed case was defined as an individual experiencing diarrhoea (two or more loose stools in a 24-h period) within 48 h after eating cheese from the implicated farm and *S. berta* isolated from their stool between 8 September and 24 October 1994. A suspect case was defined as a person who reported an acute gastrointestinal illness within 48 h of eating the implicated cheese product during the same time frame as a confirmed case. All cases were interviewed by telephone using a standard questionnaire to elicit information on clinical symptoms, burden of illness and point of purchase of the cheese. Samples of left-over cheese were collected from the homes of 14 cases. Case finding was facilitated by media coverage of the outbreak on 12 and 18 October encouraging self-reporting, and follow-up of all *S. berta* isolations reported to the CPHL during September and October.

Environmental investigation

Interviews with the cheese producers were conducted on three occasions to review the cheese production process and investigate all potential sources of contamination. The farmers were ordered, on 6 October, to halt all cheese sales until the investigation was completed. Samples of cheese and cheese curd were collected from the farm for microbiological analysis. Environmental swabs of cheese processing equipment and samples of well water and cistern water were also collected for culture. The farmers were asked to provide a stool specimen for microbiological analysis. Farm records of cheese sales were reviewed to estimate the volume sold during the outbreak period. Interviews were also conducted with three other farmers who produced the curds used to make the cheese.

Microbiological investigation

Food samples (25 g) were mixed with 225 ml of proteose peptone (1% w/v) broth and incubated for 18–20 h at 36 °C. One ml of sample suspension was

then transferred to tetrathionate brilliant green broth (Difco Laboratories, Detroit, MI, USA) and incubated for 48 h at 42 °C. A loopful of suspension was inoculated onto novobiocin brilliant green agar (Difco Laboratories) and onto brilliant green sulphur agar (Difco Laboratories) and incubated for 24 h at 36 °C. Adequacy of pasteurization of the cheese and curds was assessed using standard methods to test for residual alkaline phosphatase [2]. Water samples each containing 200 ml were filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA) and the filter was placed in 50 ml of proteose peptone (1% w/v) broth and then processed as described for the food samples. Environmental swabs were mixed with 10 ml of neutralization buffer and then diluted 1 in 10 before being processed as described for the food samples. Colonies resembling *Salmonella* species were identified using conventional methods [3]. Human stool specimens were collected and processed using conventional laboratory methods. *Salmonella* spp. cultures isolated from both human and non-human sources were serotyped at the Enteric Reference Laboratory, Central Public Health Laboratory, Toronto, Ontario.

Pulsed-field gel electrophoresis (PFGE) genomic fingerprinting was chosen as a molecular typing procedure for *S. berta* isolates for the outbreak investigation. Molecular typing of 24 isolates from human faecal specimens, cheese samples and chicken carcasses suspected to be epidemiologically associated with the outbreak was performed by analysing the genomic macrorestriction patterns generated by PFGE. A random sample of 24 cultures, unrelated to the outbreak from sporadic cases of *S. berta* and representative of the various regions of Ontario isolated during the 8-month period preceding and the 4-month period following the outbreak, were used for comparison. *S. berta* NCTC 5118 was used as a control strain.

The incorporation of bacterial cells and preparation of genomic DNA in agarose plugs were performed by using methods described previously [4]. The digestion of genomic DNA with the infrequently cutting restriction enzyme *Xba* I (New England Biolabs, Mississauga, Ontario, Canada) was carried out according to the manufacturer's recommendations. High molecular weight DNA restriction fragments were separated by using the clamped homogeneous electric field (CHEF) PFGE system (CHEF-DR III apparatus, Bio-Rad Laboratories, Mississauga, Ontario, Canada). Electrophoresis was performed for

20 h using 1% agarose gels (Pulsed field certified agarose, Bio-Rad) in $0.5 \times$ TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA [pH 8.0]) at 14 °C. The voltage gradient was 6 v/cm, the switching interval was 5–50 s and the reorientation angle was 120°. Following electrophoresis, the gels were stained with ethidium bromide (0.5 µg/ml) and the macro-restriction patterns were visualized by UV illumination. Isolates with indistinguishable patterns were considered to belong to the same type.

RESULTS

Case investigation

Of 79 clinical cases associated with consumption of the implicated cheese, 36 were laboratory confirmed with *S. berta* and 44 were suspect cases. This included 45 females and 34 males with ages ranging from 14 months to 91 years (median age 62 years). We also identified three secondary, culture-confirmed cases who had not consumed the cheese, but were contacts of confirmed cases. The greatest number of cases (82%) occurred in the region of Waterloo where the cheese was produced. The frequency of symptoms among the cases included diarrhoea (98%), bloody stools (7%), cramps (78%), fever (26%) and vomiting (22%). Illness lasted between 1 and 28 days with a median of 7 days. Sixty-two people sought medical attention and 15 people were hospitalized for their illness. Hospital stays were in the range 1–14 days (median 7.5 days). Many of the affected individuals were retired. However, 10 individuals were off work for 1–17 days (median 3 days). Confirmed cases were more likely than suspected cases to be female ($P = 0.03$, Yates's Chi Square), and to have visited an emergency room or be hospitalized ($P = 0.006$, $P = 0.003$ respectively, Yates's Chi Square). The outbreak occurred between 8 September and 24 October 1994 and was characterized by weekly increases in the number of new cases between 11 September and 3 October (Fig. 1). These increases followed weekends when the implicated cheese was sold at farmers' markets.

Environmental investigation

The implicated cheese was a specialty product known as 'cook' cheese or 'Koch Käse', traditionally eaten for breakfast spread on toast which, to the best of our knowledge, is made only in the Waterloo Region of the province. It was produced in an unregulated

manner on two different farms in the region and under proper licence at a local dairy. The implicated cheese was produced on one of the farms from skim milk curds prepared from unpasteurized milk obtained from three other farms. A review of the cheese-making processes revealed several opportunities for contamination of the final product. The cheese was made by ripening the skim milk curds at room temperature in large buckets for 2–3 days. Following ripening, salt and baking soda were added as the cheese was cooked to a paste-like consistency. Temperatures and cooking times were monitored haphazardly. The cheese was sold mainly at farmers' markets which were open on weekends, with some sales at the farm's gate.

The farmers recalled dressing four chickens on the farm on 2 September 1994 as they were finishing a batch of cheese. The carcasses were soaked in two buckets overnight, until 3 September, before being frozen for later consumption. One bucket was used for ripening cheese curds 3 days later without being properly disinfected. Two of the farmers became ill on 8 September, 24 h after sampling the new batch of cheese. Another family member became ill the next day, 9 September, after eating the cheese. The cheese from this batch was mainly sold at farmers' markets on 11 September. The second bucket was used in producing cheese sold on 17 September. The cheese-making equipment was old and difficult to clean. Disinfection and hand washing practices were not adequate to ensure the safety of the cheese.

Cheese purchased by identified cases accounted for approximately 75 of the 945 lbs (8%) sold during the outbreak period. The median amount of cheese purchased per person was 500 g or 1.10 lbs (range 250–500 g or 0.55–11 lbs). Therefore, it is possible that 789 individuals (range 79–1578) bought the unaccounted-for cheese product. In most cases, the product would have been shared with other people.

Microbiological investigation

Of 47 people with faecal specimens reported positive for *S. berta* and onset of illness between 8 September and 24 October 1994 in Ontario, 39 (83%) were primary and secondary cases associated with the outbreak. A stool culture from one of the farmers was positive for *S. berta*. This individual remained *S. berta* positive for approximately 4 months. *S. berta* was cultured from 12 of 14 left-over cheese samples obtained from the homes of cases. Four of these 14

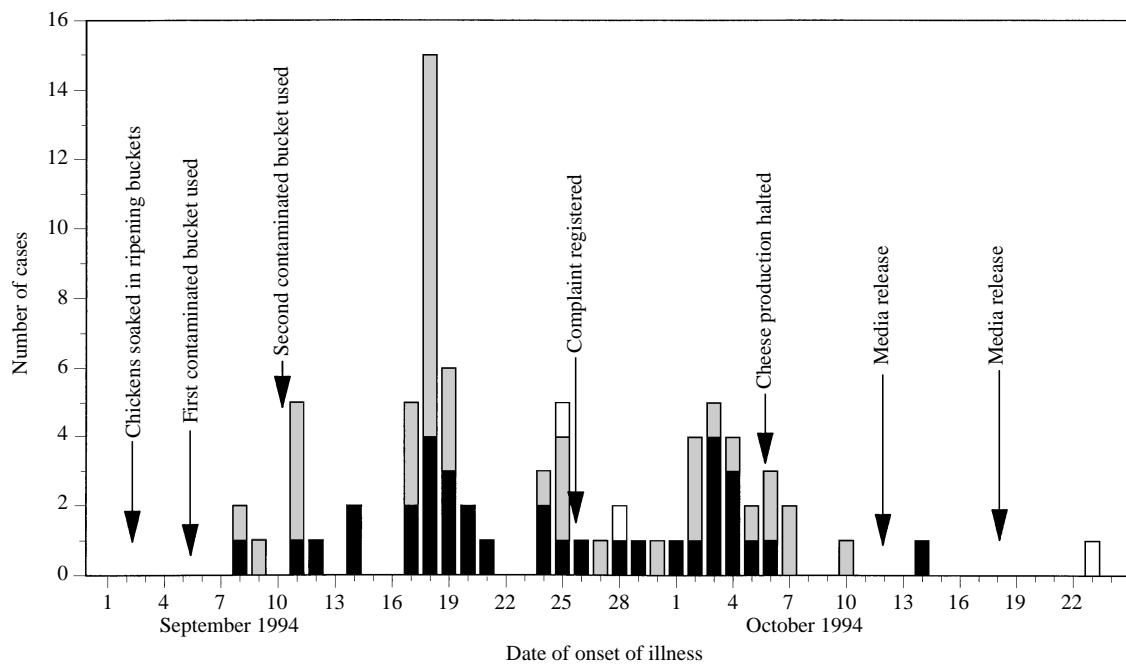


Fig. 1. Onset of illness by number of cases of *S. berta*, Ontario, Canada, 1994. Solid black bars, confirmed cases; grey bars, suspect cases; white bars, secondary cases.

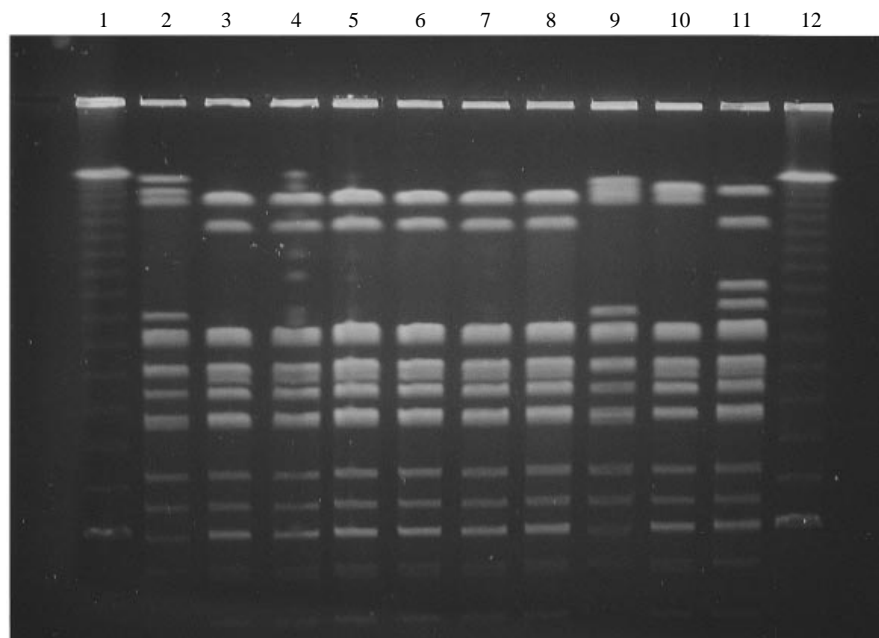


Fig. 2. PFGE patterns of *Xba* I-generated genomic fingerprints of *S. berta*, Ontario, Canada, 1994. Lanes 1 and 12, molecular size markers (bacteriophage lambda ladder). Lane 2, *S. berta* NCTC 5118 (control strain). Lanes 3–8, selected isolates from outbreak: lane 3, chicken carcass; lane 4, cheese curd; lane 5, patient A cheese sample; lane 6, patient A stool specimen; lane 7, patient B cheese sample; lane 8, patient B stool specimen. Lanes 9–11, selected non-outbreak-related isolates.

samples were also positive for phosphatase, indicating they were unpasteurized. *S. berta* was cultured from one of four cheese curd samples collected on the farm. The positive sample was taken from a bag that had been opened and partly used in a batch of cheese. This sample and one other were positive for phosphatase.

Two cheese samples collected from the farm had been re-boiled by the farmer before they were tested and both samples were negative for *Salmonella* spp. and phosphatase. Cultures of one of the chicken carcasses dressed on the farm on 2 September and subsequently soaked in a ripening bucket also yielded *S. berta*.

Well water and cistern water from the sink in the cheese-making room had high coliform counts. The cistern water also had a high count for *E. coli*; however, *Salmonella* sp. was not cultured from either source. Environmental swabs from some of the cheese-making equipment revealed a high background level of bacterial contamination, but no specific pathogens were cultured from these surfaces.

Molecular typing of 48 *S. berta* isolates was performed by analysing the genomic macrorestriction patterns generated by PFGE. Twenty-four cultures were associated with the outbreak including faecal isolates from 13 cases and isolates from 8 cheese samples obtained from these cases' homes, 1 from a cheese curd sample and 2 from chicken carcasses. Another 24 cultures, epidemiologically unrelated to the outbreak, were isolated from sporadic cases of salmonellosis that occurred in various regions of Ontario during the 8-month period preceding the outbreak (16 isolates) and during the 4-month period following the outbreak (8 isolates).

The *Xba* I macrorestriction patterns of each of the isolates associated with the outbreak were indistinguishable (Fig. 2). In contrast, 5 distinct patterns were observed among 24 epidemiologically unrelated isolates collected before and after the outbreak. Only three isolates, all from the 4-month period following the outbreak, had the outbreak strain pattern. The patterns of selected non-outbreak-related isolates are also shown in Figure 2.

Based on the investigation of the cases and the farm, and bacterial culture results, the farm was ordered to destroy all remaining cheese and curds on the premises and not engage in such activity in the future unless it was done in a licensed facility.

DISCUSSION

This outbreak may have gone undetected had it not been for the relatively uncommon serotype and unusual food product involved. Recognition of this outbreak was facilitated by passive laboratory-based surveillance by the CPHL of serotypes of *Salmonella* isolates submitted by various clinical laboratories and the initiative of a consumer to alert regional public health authorities of a potentially unsafe product. The environmental investigation revealed that cross-contamination of a soft cheese product produced on a single farm led to a community outbreak of *S. berta*. The epidemic curve is consistent with this finding, showing increases in cases at weekly intervals, approxi-

mately 24 h after the days when the farmers' markets were open and the implicated cheese was sold. We believe the source of the cheese contamination to be the chicken carcasses which had been soaked in buckets used to ripen the cheese curd. One of the farmers also was infected with the outbreak strain of *S. berta* after eating their product and remained positive for several months. Therefore, what started as a problem of cross-contamination at processing, could have been perpetuated by an infected food worker. A small proportion of the curd and cheese samples tested were positive for phosphatase indicating improper pasteurization. The poor sanitation practices leading to cross-contamination and inadequate pasteurization methods illustrate the need for strict regulation of home-based food producers.

A case-control study was not conducted as part of this outbreak investigation because of strong evidence available early in the investigation implicating the 'cook' cheese and the unsanitary conditions under which it was produced. Recall of consumption of this cheese was very accurate since it is a unique and uncommon product only available in one region of the province from a few markets or the farm. People also recalled very specific information which confirmed the farm involved such as physical descriptions of the farmers who sold it and the location of the booth in the market where they purchased it. A case-control study would have been useful in ruling out other possible sources of infection and in estimating the magnitude of the association between the cheese and illness. However, when the association between the chicken and the cheese was learned, the focus of the investigation shifted to preventing this type of outbreak in the future and molecular typing using PFGE to prove the link between the cases and the cheese.

Cheese is a relatively uncommon vehicle for human salmonella infection but several outbreaks have been reported in recent years [5–9]. In 1985, an outbreak of *S. typhimurium* in Switzerland was traced to the consumption of a soft cheese product made from raw milk [5]. Two extensive outbreaks of salmonellosis in North America, one caused by *S. heidelberg* [6] and the other caused by *S. typhimurium* [7] were traced to deficiencies in pasteurization procedure for milk used in the production of cheddar cheese. An outbreak of *S. javiana* and *S. oranienburg* in the US was found to be associated with mozzarella cheese contaminated by environmental sources or infected production workers

[8]. In addition, an outbreak of *S. typhimurium* in Italy was associated with cross-contamination of mozzarella cheese with raw chicken [9]. The high fat content of cheese may protect bacteria from human gastric acidity which increases the risk of human infection from contaminated cheese products [10]. The findings of the present study provide further evidence for the potential role of cheese products as vehicles for foodborne bacterial pathogens.

During the last decade, an increase in the incidence of human *S. berta* infections has been reported in European countries and several studies have indicated that broiler chickens are the major source of human infections [11, 12]. A similar increase in human *S. berta* infections has not been observed in Canada; this serovar did not rank among the 10 most common serotypes isolated from humans in 1993 [1]. A nationwide survey of broiler flocks in Canada found *S. berta* in environmental samples from 4.8% of farms tested, making this serotype the sixth most common serotype identified from this source [13]. This outbreak illustrated how this organism can quickly spread from broiler chickens to humans, and in this case result in a community outbreak. The magnitude of the outbreak was probably much larger than reported since a great deal more cheese was sold than was accounted for by the interviewed cases (8% of total). Although contamination would not likely be homogeneously spread through every batch, it is probable some of it was also contaminated. Many mild cases may not have sought medical attention or called the health unit to report their illness. Several suspected cases who reported visiting a physician did not submit a stool sample for culture.

PFGE macrorestriction pattern analysis has been used as a molecular typing procedure for several *Salmonella* serotypes [14–17]. However, to the best of our knowledge, the present study represents the first use of PFGE to characterize *S. berta* strains. This outbreak afforded the opportunity to demonstrate the usefulness of PFGE in linking the suspect vehicle and source of contamination to outbreak cases. While the PFGE results for this investigation were not done in time to influence the regulatory actions taken against the farm, the evidence provided strong information to support them. All *S. berta* isolates suspected of being associated with the outbreak had indistinguishable macrorestriction patterns, supporting the environmental evidence linking these isolates to the outbreak. In contrast, 5 distinct patterns were identified among 24 epidemiologically unrelated strains. The pattern of

the outbreak strain was identified in 3 of 8 isolates identified following the outbreak, evidence that the outbreak introduced a new strain into the community and that continued transmission of this strain occurred in the period following the outbreak. PFGE enables investigators to detect minor genetic differences between isolates of the same species and is quickly becoming the gold standard for confirming epidemiological links in outbreak investigations.

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