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Molecular epidemiology of an outbreak caused by *Salmonella enterica* serovar Newport in Finland and the United Kingdom

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SUMMARY

Between December 1997 and January 1998 an increase in the number of isolates of *Salmonella enterica* serovar Newport, a serotype rarely causing indigenous infections in Finland, was detected. This included two clusters of gastroenteritis following funeral meals. An inquiry via Enter-net revealed a concomitant increase in cases of *S.* Newport in the United Kingdom. To investigate the Finnish outbreak, a total of 56 *S.* Newport strains (22 from the outbreak period, 27 from pre- and post-outbreak period, and 7 from imported food producing animals) were studied by pulsed-field gel electrophoresis (PFGE); selected isolates were also phage typed. Two retrospective questionnaire studies evaluating food exposures among the funeral attendants were conducted. All isolates from the clusters had an identical PFGE pattern which was also found in 13 infections temporally close to but not associated with the clusters. The Finnish outbreak was caused by the same phage type as the one in the United Kingdom. In both clusters, an epidemiological link between illness and exposure to cured ham was found. In conclusion, the outbreak was not limited to the two clusters but was more widely spread both in and outside Finland. Early alarm systems of food-borne outbreaks and collaboration between European countries are needed for investigating international outbreaks.

INTRODUCTION

Non-typhoid *Salmonella enterica* is a leading cause of foodborne bacterial infections in Finland, in the United Kingdom, and worldwide [1]. However, when compared to many other European countries the annual number of reported cases in Finland is rather low [2]. In 1996, 2 years after Finland joined the European Union and barriers for animal and food trade were removed, the incidence of human salmonellosis was 54 cases per 100000 inhabitants, and most (81 %) cases were associated with foreign travel. The low incidence of indigenous salmonellosis as well as the low prevalence of salmonellae in the domestic food chain possibly reflects the efficacy of the Finnish National Salmonella Control Programme.

S. enterica serovar Newport is a relatively uncommon cause of salmonellosis in Finland; in 1996 it accounted for only 1·3 % of all human salmonellosis. The majority of findings of this serotype have been associated with foreign travel (Fig. 1). Furthermore, there are no known reservoirs for this serotype in Finnish food production animals.

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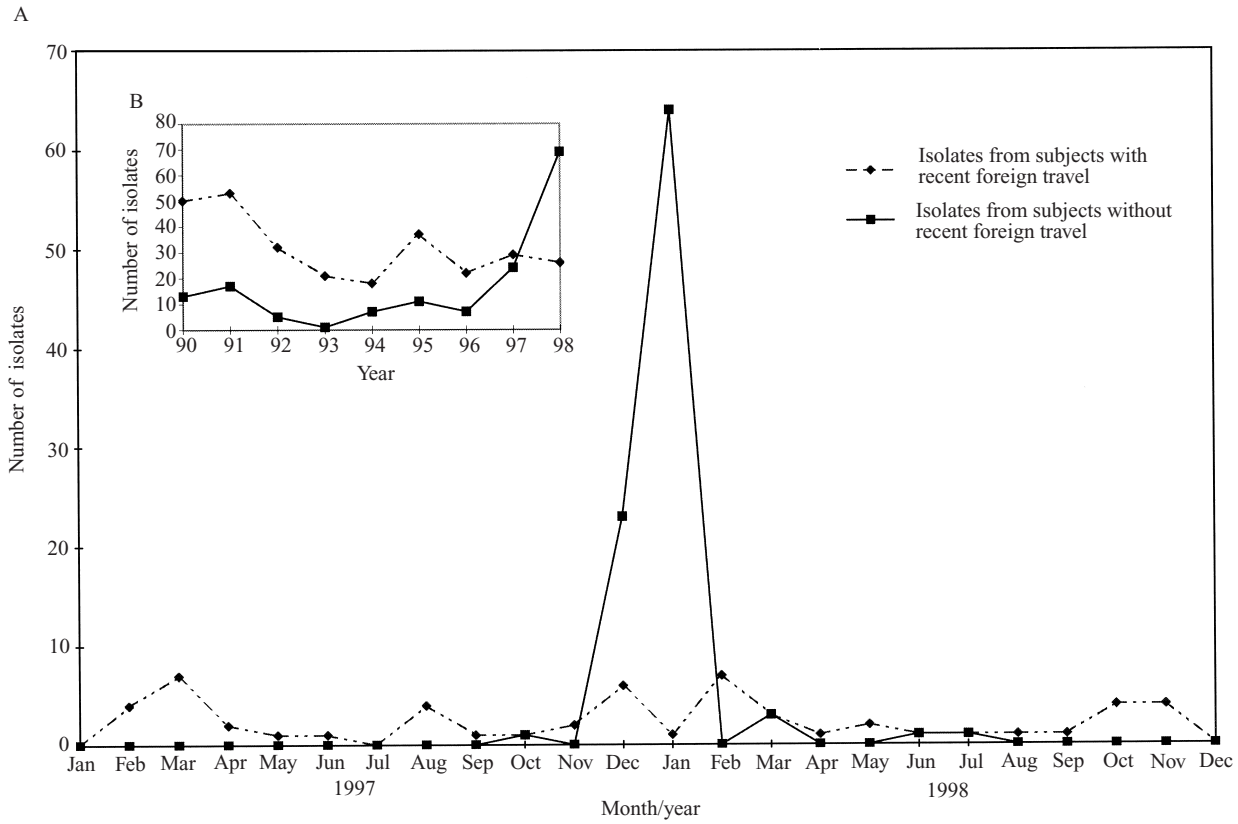

Fig. 1. Number of *Salmonella enterica* serovar Newport isolations by year in 1990–8 and by month in 1997–8, Finland.

From December 1997 to January 1998 a rise in the number of *S*. Newport isolates was observed. An inquiry via Enter-net, a network for communicating information on salmonella among European Union health authorities [3], revealed a simultaneous increase in cases of *S*. Newport in the United Kingdom. In order to find the possible source and epidemiology of the outbreak, the restriction fragment length polymorphism of chromosomal DNA after pulsed field gel electrophoresis (PFGE) of outbreak and non-outbreak human and non-human *S*. Newport isolates was studied. A subset of the isolates were also phage typed. The results obtained from studies with the Finnish outbreak strains were compared with those of British outbreak isolates. We describe here the results of the bacteriological investigations as well as the epidemiological investigations on the food vehicle implicated in the Finnish infections.

MATERIAL AND METHODS

Background

The Finnish microbiological laboratories report all salmonella isolations to the National Infectious Disease Register at the National Public Health Institute (KTL). All isolates from infections without recent foreign travel and a large proportion of the strains from infections potentially acquired abroad are sent to the Laboratory of Enteric Pathogens (LEP) at KTL for serotyping. The surveillance and reporting of food- and water-borne outbreaks includes a notification telefaxed by local health or environmental authorities to the Department of Infectious Disease Epidemiology at the KTL, as soon as suspicion arises. The two initial local outbreaks of *S*. Newport were associated with funeral receptions, arranged by different catering services, one in northern (Ylikiiminki) and another in southern Finland (Hausjärvi).

Epidemiological investigation

Two independent retrospective cohort studies were conducted, one in Ylikiiminki and the other in Hausjärvi. The questionnaire was distributed by mail to all persons who had attended the funeral receptions (Ylikiiminki, 113; Hausjärvi, 50). The questionnaire gathered information about the onset, duration, and characteristics of symptoms since the funeral reception, demographics, and food eaten in the funeral

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Table 1. Salmonella enterica *serovar Newport strains examined by PFGE*

Date/month of isolation	Source of isolation	Country/geographic origin	PFGE pattern
2/1995	Human	Finland, Kotka	26
5/1995	Human	Finland, Helsinki	25
6/1995	Human	Finland, Hyvinkää	24
7/1995	Imported turkey	Ireland	27
9/1995	Human	Finland, Helsinki	22
9/1995	Human	Finland, Uusikaupunki	23
11/1995	Human	Finland, Laukaa	21
11/1995	Imported turkey	Unknown	NT
1/1996	Imported duck	Unknown	19
3/1996	Human	Finland, Karvia	14
3/1996	Human	Finland, Tampere	15
3/1996	Human	India*	18
3/1996	Imported turkey	France	20
4/1996	Human	Finland, Tampere	13
5/1996	Human	Finland, Kurikka	11
5/1996	Human	Finland, Porvoo	12
5/1996	Human	Germany*	NT
5/1996	Imported turkey	France	NT
7/1996	Human	Kenya*	NT
9/1996	Human	Turkey*	NT
10/1996	Human	Tunisia*	17
12/1996	Human	Morocco*	16
2/1997	Imported horse	USA	9‡
2/1997	Imported horse	USA	10‡
14/2/1997	Human	Tunisia*	NT
21/2/1997	Human	Spain*	8
18/6/1997	Human	Turkey*	7
6/8/1997	Human	Tunisia*	NT
21/8/1997	Human	Turkey*	6
27/10/1997	Human	Finland, Vantaa	5
11/11/1997	Human	Spain, Canary Islands*	1
19/12/1997	Human	Finland, Helsinki	1
22/12/1997	Human	Finland, Iitti	1
26/12/1997	Human	Finland, Hausjärvi	1
29/12/1997	Human	Finland, Järvenpää	1
30/12/1997	Human	Finland, Karjaa	4
30/12/1997	Human	Finland, Hausjärvi	1
30/12/1997	Human	Finland, Espoo	1
1/1/1998	Human	Finland, Hyvinkää	1
2/1/1998	Human	Finland, Hausjärvi	1
2/1/1998	Human	Finland, Artjärvi	1
5/1/1998	Human	Finland, Parikkala	1†
7/1/1998	Human	Finland, Hausjärvi	1
7/1/1998	Human	Finland, Oitti	1
12/1/1998	Human	Finland, Salo	1
12/1/1998	Human	Finland, Hyvinkää	1
13/1/1998	Human	Finland, Ylikiiminki	1
14/1/1998	Human	Finland, Hamina	1
15/1/1998	Human	Finland, Helsinki	1†
19/1/1998	Human	Finland, Ylikiiminki	1
19/1/1998	Human	Finland, Ylikiiminki	1†
20/1/1998	Human	Finland, Ylikiiminki	1
25/1/1998	Human	Finland, Helsinki	1
16/3/1998	Human	Finland, Virtasalmi	1
23/6/1998	Human	Finland, Pori	3
29/7/1998	Human	Finland, Muijala	2

* Isolated in subjects recently returned from abroad.
† All of the same phage type ($n = 3$).
‡ Each of different phage type ($n = 2$).
NT, not typable.

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receptions. In Ylikiiminki, persons had to have attended the funeral reception on 14 December 1997, and subsequently had diarrhoea to meet the case definition. In Hausjärvi, persons had to have attended the funeral reception on 20 December 1997 and subsequently have a stool culture positive for *S.* Newport to meet the case definition. Univariate statistical analysis of the categorical data was performed using the χ^2 and Fisher's exact test, as appropriate. Confidence intervals (CI) for relative risks (RR) were calculated using Epi-Info.

S. Newport strains

Salmonella serotyping was carried out by standard techniques [4]. A total of 56 *S.* Newport isolates were selected for further studies (Table 1). Of these, 22 isolates (4 representing the cluster in Ylikiiminki, 4 in Hausjärvi, and 14 sporadic isolates from Finland) were from the outbreak period (i.e. December 1997–January 1998). Each year a representative set of isolates from salmonella cases acquired indigenously or abroad had been stored at −70 °C in sterilized skimmed milk. As controls, 27 isolates (12 from subjects with recent travel and 15 without recent travel) obtained before and after the outbreak period in 1995–8 were studied. In addition, seven isolates from imported food production animals obtained in 1995–7 were included in the study.

PFGE

S. Newport isolates were grown overnight on nutrient agar at 37 °C. Bacterial cells were suspended in 1200 μl of TEN (0·1 M Tris–HCl, 0·15 M NaCl, 0·1 M EDTA, pH 7·5) until the absorbance of the 1:10 diluted suspension reached 0·150–0·200 at a wavelength of 600 nm. Agarose plugs were prepared by mixing equal volumes of the bacterial suspension and 2% molten LMP-agarose (Sea Plaque agarose, FMC BioProducts, Rockland, Maine, USA) and pipetting the mix into plug moulds on ice. The solidified plugs were gently shaken in 3 ml of lysis buffer (0·5 M EDTA, 1% *N*-lauroylsarcosine, 1 mg/ml proteinase K) overnight at 56 °C. The lysis buffer was removed and the plugs were washed first in 8 ml, of TE (10 mM Tris–HCl, 1 mM EDTA, pH 8·0) buffers, then in 5 ml of TE containing 4 mM PMSF (phenyl methyl sulphonyl fluoride), and finally three times in 8 ml of TE buffer. The minimum standing time in room temperature was 30 min per wash. The plugs were stored at 4 °C in TE buffer. For isolates which were not typable with the method described above, additional procedures were implemented to inactivate DNase activity: (i) plugs were incubated overnight at 37 °C in lysozyme solution (6 mM Tris–HCl, 0·1 M EDTA, 1 M NaCl, 0·5% Brij 58, 0·2% Na-deoxycholate, 0·5% sodium lauroyl sarcosine, 1 mg of lysozyme per ml) prior to the deproteination; (ii) the concentrations and incubation times of lysozyme (1 mg/ml overnight) and proteinase K (0·15 mg/ml overnight) were progressively increased to 2 mg/ml for 24 h and to 2 mg/ml for 48 h, respectively [5]; (iii) harvested bacterial cells were treated with formaldehyde by both original [6] and modified [7] methods. For restriction endonuclease digestion, a 1 mm section was cut from each plug and equilibrated in 100 μl of restriction buffer for 30 min. The buffer was replaced with 10 U of *Xba*I (Boehringer–Mannheim, Germany) in 100 μl fresh restriction buffer and the plugs were incubated overnight at 37 °C. The digested plugs were inserted into 1% agarose D-5 gel (Pronadisa, Spain) in 0·5 × TBE (45 mM Tris-borate, 1·0 mM EDTA) running buffer. Electrophoresis was performed on CHEF-DR II systems (BioRad, California, USA) for 24 h at 14 °C, pulse ramp-time 5–70 s, 6·3 V/cm. The gels were stained for 40 min with ethidium bromide (1 mg/ml) in 0·5 × TBE, rinsed in water for 20 min and photographed under UV illumination with Polaroid film. Similarity of strains was assessed according to the guidelines of Tenover [8].

Phage typing

A total of 20 *S.* Newport isolates (5 of which were included in the PFGE analysis, Table 1) were typed using a *S.* Newport phage-typing scheme consisting of 12 typing phages (L. Ward, unpublished observations) developed in the Laboratory of Enteric Pathogens, London. Sixteen isolates represented the Ylikiiminki and Hausjärvi clusters, four were from imported meat (horse meat, turkey) and one from fertilizer.

Environmental investigations

All leftover foods from the funeral receptions were cultured for salmonellae at the local food laboratories. The trace-back of suspected food was done by the national food safety authorities and the production processes by the local food safety authorities.

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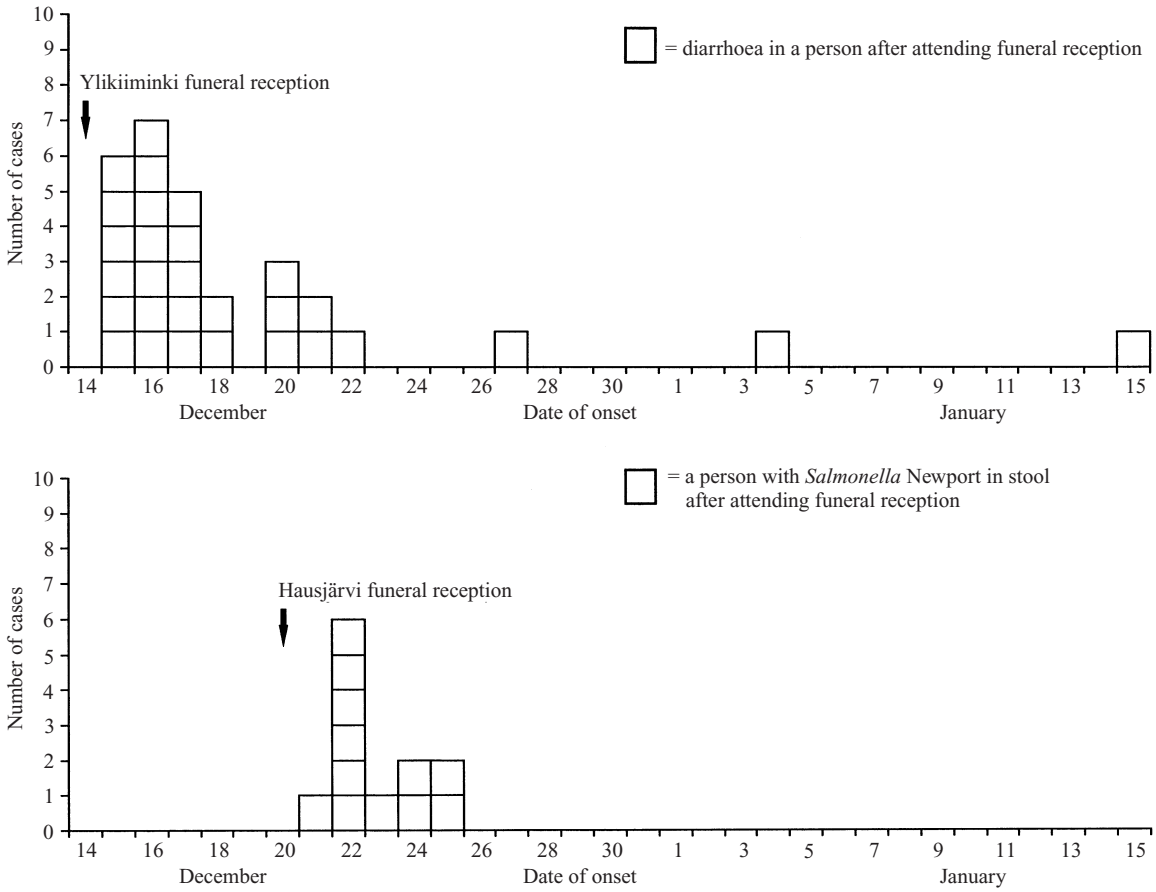

Fig. 2. Number of cases with diarrhoea by symptom onset among persons who attended the funeral receptions in Ylikiiminki and Hausjärvi.

RESULTS

Epidemiological investigation in Ylikiiminki

One hundred and nine (96%) of the 113 persons who attended the funeral reception in Ylikiiminki completed the questionnaire. Thirty-four (31%) met the case definition, 18 of them had a positive stool culture for *S.* Newport. Symptoms reported by the case-persons in addition to diarrhoea (100%) were abdominal pain (94%), nausea (44%), and fever (42%). For 29 case-persons the onset date of symptoms was available. The first cases became ill on 15 December 1997 and the final ones in January 1998, most (62%) of them occurred within 3 days following the reception (Fig. 2). The persons who reported having eaten white bread with ham met the case definition four times more likely than those who had not (40 *vs.* 10%; RR 4·0; 95% CI 1·3–12·2; $P = 0{\cdot}006$). The consumption of dark dry cake was also associated with illness (48 *vs.* 21%; RR 2·3; CI 95% 1·3–4·2; $P = 0{\cdot}007$). However, of the 34 cases, 29 could be explained by the consumption of white bread with ham whereas only 19 were associated with consumption of the dark dry cake (85 *vs.* 56%). One food handler had a stool sample positive for *S.* Newport. Whether this food handler had eaten the food served at the reception and/or prepared the ham sandwiches is not known.

Epidemiological investigation in Hausjärvi

Forty-four (88%) of the 50 persons who had attended the funeral reception in Hausjärvi completed the questionnaire and 38 (76%) submitted a stool sample. Twenty-two persons (58%) met the case definition (i.e. stool sample positive for *S.* Newport). Most common symptoms reported by the cases were diarrhoea (59%), abdominal pain (59%), and fever (41%). For 12 cases with diarrhoea the date of onset of symptoms was available. The first cases fell ill with diarrhoea on 21 December 1997 and the last cases on 25 December 1997 (Fig. 2). Most (67%) cases fell ill within 3 days of the reception. Of the 28 food items served at the ceremony, only the consumption of cured ham was associated with meeting the case definition (75 *vs.* 25%; RR 3·0; 95% CI 1·1–8·2; $P = 0{\cdot}012$). One of the four food handlers had a stool

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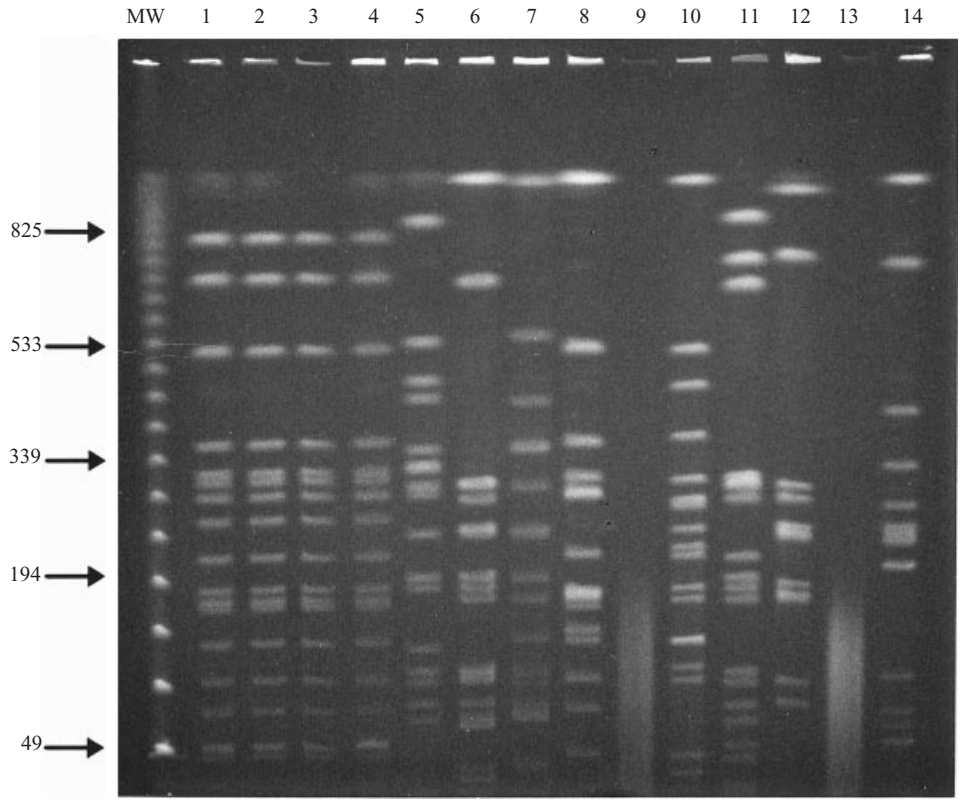

Fig. 3. PFGE patterns of representative isolates of *Salmonella enterica* serovar Newport. Lanes 1–4, human isolates from the outbreak period December 1997 to January 1998 (1 from Hausjärvi and 3 from other parts of Finland); lanes 5–8, human isolates from the pre-outbreak period 1995–7; lanes 9–12, human isolates from subjects recently returned from Spain, Turkey, Tunisia, and Marocco in 1996–7; lanes 13, 14, isolates from imported turkey from France and Ireland in 1995–6. MW is the DNA molecular-size standard (lambda ladder) and numbers on the right-hand side are molecular size markers in kilobases. The enzyme used for DNA digestion was *Xba*I.

culture positive for *S.* Newport. This food handler had symptoms of mild gastrointestinal illness before Christmas, had not eaten the food served at the funeral reception and had not prepared the cured ham rolls.

Phenotypes and genotypes of the isolates

Of the 56 isolates, 49 were typable by PFGE and showed 27 distinct PFGE banding patterns. Seven isolates were not typable with any of the tested methods due to their pronounced production of extracellular DNAses, which caused DNA degradation during its isolation. The PFGE banding patterns of all isolates tested from the Ylikiiminki and Hausjärvi clusters were indistinguishable (i.e. pattern 1, Table 1 and Fig. 3). The same pattern was also seen in 15 isolates obtained from subjects living in other parts of Finland and all but 2 were from the outbreak period. Of these subjects, only one had a recent travel history (Canary Islands). Most (25/27) of the control isolates obtained before and after the outbreak period as well as those from the food production animals produced their own distinct patterns. All 16 isolates from Ylikiiminki and Hausjärvi were phage type 30. The 4 non-human isolates had 4 different phage type patterns and were different from the outbreak isolates.

Environmental investigations

All leftover food items were negative for salmonellae. The ham was not available for culture either in Ylikiiminki or in Hausjärvi. The hams were supplied by two different producers. The pork was of Finnish and Danish origin. One of the two producers had imported frozen turkey from the United Kingdom. No defects in the production processes were detected. *S.* Newport had not been isolated in the routine internal control run by these factories, including staff screening.

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DISCUSSION

The investigations by molecular and phenotypic methods showed that the increase in number of *S.* Newport isolates detected between December 1997 and January 1998 was due to a single strain. The epidemiological studies of two point-source clusters demonstrated an association with cured ham consumption. In addition, Enter-net revealed a concomitant increase in *S.* Newport cases in the United Kingdom, the results of phage typing suggesting that the outbreaks in Finland and United Kingdom were caused by identical strains.

People are increasingly travelling on holidays in foreign countries and many food items are crossing national borders. Consequently, salmonella outbreaks are likely to cross national boundaries. The proportion of reported cases related to foreign travel varies considerably across Europe [1]. However, in contrast to most other European countries a majority of Finnish patients have acquired their infections abroad and the indigenous level is comparatively low, as in Norway and Sweden [9, 10].

Based on the results of the molecular and epidemiological investigations, at least 70 persons were infected in Finland during this outbreak caused by *S.* Newport. The true size of the outbreak was probably much bigger, as we can assume that many patients with diarrhoea do not seek medical care at all, only a minority of patients with diarrhoea have a stool culture taken, and not all isolates of salmonella are sent by the microbiology laboratories for serotyping.

Two independently designed retrospective cohort studies were conducted in different parts of Finland which demonstrated that *S.* Newport infections were only associated with the consumption of ham. The epidemic curves from both clusters were consistent with a point source outbreak and the incubation period is consistent with infection with salmonella. Some cases with late symptom onsets could have been secondary cases, as there were several family members from the same family who attended the funeral receptions. In both clusters, a food handler also had a positive stool culture. It is more likely that the food handlers were victims of a foodborne infection than the source of it, a hypothesis increasingly presented in the literature [11]. Unfortunately, implicated batches of ham served at the two receptions were not available for culture, and the specific mechanism for cured ham contamination could not be identified.

Isolates from 15 additional persons, who did not attend the funeral receptions, produced an identical PFGE pattern with the strains from the two clusters. These persons lived in various parts of Finland. As in other large food produce-related outbreaks, this scattered geographical pattern probably reflects the wide distribution of the contaminated product, in this case cured or another food item that cross-contaminated cured ham. *S.* Newport isolates from Finnish and UK patients had the same phage type which suggests international spread. However, no epidemiological or trace-back information was available on the UK patients and in Finland the trace-back of implicated lots of ham, their production process, and the origin of the pork did not reveal a probable source.

All *S.* Newport isolates from the two point-source clusters had indistinguishable PFGE patterns after *Xba*I digestion of genomic DNA. The same PFGE pattern was also found among almost all (13/14) other isolates obtained during the outbreak period, while most (25/27) of the typable isolates obtained before and after the outbreak period had their own distinct patterns. Because of the diversity of the PFGE patterns with the use of this restriction enzyme, the appearance of an indistinguishable patterns is strong evidence for the presence of a common contaminating source. Furthermore, all outbreak isolates examined were easily typable by PFGE in contrast to the few strains representing diverse origin; they were not typable because of their excessive production of DNases.

Our study shows the value of efficient surveillance and outbreak detection methods in identifying widely dispersed outbreaks that may otherwise be missed. This outbreak was detected quickly because of timely serotyping of salmonella isolates by the national public health reference laboratory. The recent application of a new outbreak reporting system for food- and water-borne outbreaks facilitated the prompt epidemiological investigation of the two clusters with the collaboration of the local and national authorities. The inquiry via Enter-net further allowed to link these two national outbreaks together. However, the study also illustrates the complexity of national and international communication between public health experts currently needed in the management of such outbreaks, especially in analytical studies and tracing back the suspected food [12]. Widely dispersed foodborne outbreaks are likely to pose an increasing

challenge to public health, as food is increasingly produced in large-scale industrial facilities and widely distributed.

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