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Investigation of increased listeriosis revealed two fishery production plants with persistent *Listeria* contamination in Finland in 2010

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SUMMARY

In 2010, a marked increase in listeriosis incidence was observed in Finland. *Listeria monocytogenes* PFGE profile 96 was responsible for one-fifth of the reported cases and a cluster of PFGE profile 62 was also detected. Investigations revealed two fishery production plants with persistent *Listeria* contamination. It appears likely that the plants were at least partly responsible for the increase of listeriosis. Epidemiological investigation revealed that 57% (31/54) of cases with underlying immunosuppressive condition or medication reported eating gravad or cold-smoked fish. Two public notices were issued by THL and Evira informing which groups were most at risk from the effects of listeriosis and should therefore be cautious in consuming certain products. Systematic sampling of foods and adequate epidemiological investigation methods are required to identify the sources of *Listeria* infections. Continuous control measures at fishery production plants producing risk products are essential.

Key words: Control, foodborne infections, *Listeria*, outbreaks, typing.

INTRODUCTION

Listeriosis, caused by the Gram-positive bacterium *Listeria monocytogenes* (*Lm*), has an average incidence of 3–4 cases per year per million inhabitants in the EU, and is associated with high case fatality [1]. The majority of cases occur in highly susceptible populations: pregnant women, newborns, adults with weakened immune systems, and the elderly ($\geqslant$65 years). Most cases are sporadic and cannot be linked to any specific source [2]. In two previous outbreaks in Finland, the vehicle for listeriosis was identified: vacuum-packed cold-smoked rainbow trout in 1997 [3] and butter in 1998/1999 [4].

In Finland, the incidence of listeriosis is comparable to that of other industrialized countries [2]. When comparing surveillance data, it should be taken into

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consideration that case definitions, diagnostic practices, and surveillance systems may vary between countries [5]. During the 2000s, the annual number of reported listeriosis cases has increased in several countries in Europe [5–8]. In March 2010, a marked increase in a certain PFGE profile of *Lm* serotype 1/2a (*Lm*96) was observed during laboratory surveillance at the National Institute for Health and Welfare (THL). An outbreak investigation that included epidemiological, traceback and laboratory investigations was initiated to determine the source of the outbreak in order to prevent further cases and outbreaks caused by *Lm*96.

METHODS

Listeriosis surveillance

Since 1995, clinicians and clinical microbiology laboratories have been obliged to report culture-confirmed listeriosis cases to the National Infectious Disease Register (NIDR) maintained by THL, and to submit isolates from patients to THL. Data on sex, age, place of residence, institution, isolation site of the bacteria and sampling date are reported. The case definition for listeriosis is in line with the definition of a culture-confirmed listeriosis case as issued by the European Commission: *Lm* is isolated from a normally sterile tissue, fetus, stillborn or newborn, or the mother.

The cases notified in 1995–2010 were classified as septic or meningitis cases depending on the isolation site. A materno-fetal case included pregnancy-associated listeriosis or listeriosis in a newborn. Infection in both the mother and newborn was counted as a single case. For cases notified in 2010, data on underlying conditions and medications were collected from medical records requested from the attending physicians. Mortality data were obtained from the Finnish Population Registry. To estimate the case fatality, deaths within 30 days of sampling were considered listeriosis related.

Questionnaire study and international outbreak inquiry

For cases notified in 2010, family members or hospital staff were interviewed. Since *Lm*96 had previously been found in fishery products and in patients who had consumed cold-smoked or gravad salmon, the questionnaire focused on consumption of cold-smoked and gravad fish products and other ready-to-eat fish products within 2 months prior to the sampling date. The brand and product names of the 13 most commonly consumed fish products in Finland were included in the questionnaire in order to find links between the listeriosis cases and specific production plants. Statistical analyses were performed using Stata v. 10.0 (Stata Corp., USA) and R v. 2.14.0 (R Development Core Team) software programs. Incidence rate ratios (IRR) were used to compare incidences in different patient groups. The population count that was used as a nominator was obtained from Statistics Finland. An international outbreak inquiry was conducted via the European Centre for Disease Prevention and Control in order to chart the incidence of *Lm*96 infection and possible sources in other European countries.

Monitoring of *Lm* in foods

It is mandatory for local official food control laboratories to send *Lm* isolates from food samples taken within the framework of any statutory control programme or from food or waterborne outbreak investigation to the national reference laboratory at the Finnish Food Safety Authority Evira for further characterization (Food Act 23/2006, Decree 1365/2011). Within the framework of the national monitoring of zoonoses and zoonotic agents (Directive 2003/99/EU), national surveys to estimate the presence and levels of *Lm* especially in vacuum-packed smoked or gravad fish products have been performed regularly. In 2010, ready-to-eat foods were monitored for *Lm.* All food *Lm* isolates detected in these surveys are stored at the national reference laboratory.

Microbiological methods

The patient isolates were serotyped at THL and the isolates from the ready-to-eat food monitoring at Evira. The isolates were confirmed as *Lm* using standard biochemical tests. Pulsed-field gel electrophoresis (PFGE) standardized by PulseNet [9] with the restriction enzyme *Asc*I (New England Biolabs, USA), was used to genotype the strains. The PFGE profiles were analysed using BioNumerics software v. 5.1 (Applied Biosystems, USA), and profiles found in patients and food samples were compared. The isolates from patients were O- and H-serotyped by slide and tube agglutination methods, respectively, using commercially available antisera (Denka Seiken Co. Ltd, Japan) as described previously [10].

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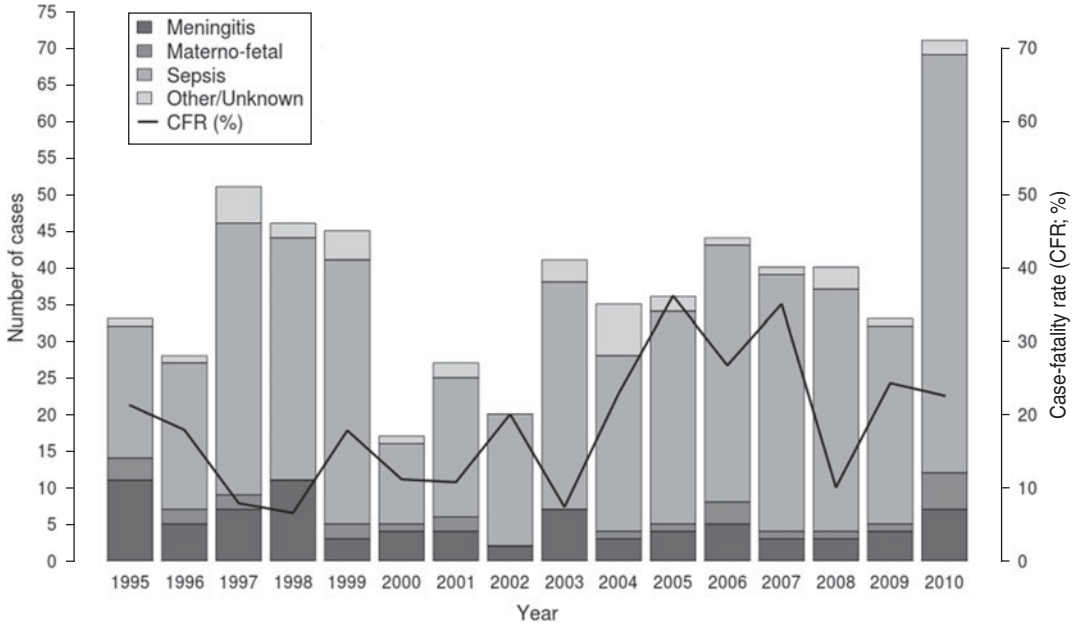

Fig. 1. Number and clinical manifestations of listeriosis cases between 1995 and 2010.

RESULTS

Listeriosis surveillance, patient interviews and characteristics of patient isolates

In 2010, 71 listeriosis cases (incidence of 13 per million inhabitants) were reported in Finland (Fig. 1). The incidence was nearly twofold [IRR 1·9, 95% confidence interval (CI) 1·5–2·5] compared to that for the period 1995–2009, when about 36 cases/year (range 18–51 cases/year, incidence 3–10 per million) were notified. Sporadic cases were reported throughout the country, with males and females equally represented. The median age of cases was 69 years, and the highest incidence of listeriosis (60 per million) occurred in those aged >70 years. This is in line with the incidence in 1995–2009 when cases were clearly more common in those aged >70 years (incidence of 9–44 per million) compared to other age groups (IRR 7·3 95% CI 6·3–8·6). In 2010, the number of septic infections increased (IRR 2·0, 95% CI 1·6–2·7; Fig. 1). Of the cases, five (7%) were materno-fetal cases.

Data on underlying conditions were obtained for 67 cases. Of these, 50 (75%) had an immunosuppressive illness (haematological malignancies 17, diabetes 16, alcoholism four cases) and/or immunosuppressive medication (29 cases), and four were pregnant. In 2010, the case-fatality rate was 23%, while the mean yearly case-fatality rate in 1995–2009 was 18%.

Of the 71 cases, 67 (94%) were interviewed. Of those, 41 (61%) remembered consuming or reported a habit of eating gravad or cold-smoked fish, but only nine could remember the brand names of the consumed products. One case never consumed fish due to allergy. Of the 54 cases that had an underlying immunosuppressive condition or medication, 31 (57%) reported consumption of gravad or cold-smoked fish. Of the cases with haematological malignancies, 12 (71%) had consumed gravad or cold-smoked fish, while one of the pregnant cases had done so.

Lm isolates from 69 cases were submitted to THL for further characterization. Serotypes 1/2a (50 strains) and 4b (16 strains) accounted for most of the cases, as was the case for the previous 10 years (Fig. 2). Among the 69 isolates, 35 PFGE profiles were found. The most common profile was *Lm*96, which was found in 13/69 samples (19%). The *Lm*96 cases were geographically dispersed, while in 11 (92%) cases there was an underlying immunosuppressive condition or related use of medication. Twelve *Lm*96 cases were interviewed but only six reported consumptions of gravad or cold-smoked fish.

The enquiry via the Food and Waterborne Diseases Network revealed that *Lm*96 was identified in several European countries between January 2009 and May 2010. It was a common PFGE profile in Europe and had been isolated from various foods, not only from fishery products.

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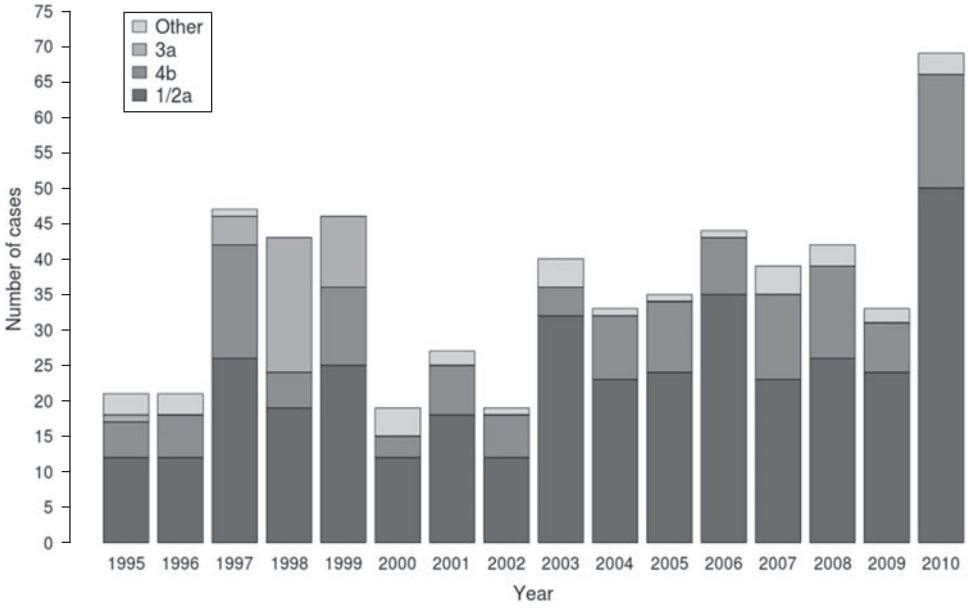

Fig. 2. Serotypes of *L. monocytogenes* strains isolated from listeriosis cases between 1995 and 2010.

Lm in foods

In 2010, Evira received 323 *Lm* isolates originating from 240 food samples in 21 local, official food control laboratories. Most of the samples (186/240, 78%) were from fishery products. Half of the isolates (162/323, 50%) originated from two fishery production plants in which persistent *Listeria* strains were detected. As part of an official survey, 257 samples from vacuum-packed cold-smoked or gravad fishery products, soft or semi-soft cheeses and vacuum-packed heat-treated meat products were analysed for *Lm* at the end of their shelf-life in 2010. *Lm* was detected only in fishery product samples (20/126, 16%). In one product, the level of *Lm* was >100 c.f.u./g [11]. In all, 307 *Lm* isolates from food were genotyped by Evira in 2010. The isolates originated from 204 food samples that were taken from food products produced in 22 production plants. Most (162, 79%) of these samples were from fishery products. Among the 307 isolates, 48 PFGE profiles were detected and *Lm*96 was the most common PFGE profile in the food samples in 2010 (37%, 76/204 samples). *Lm*96 was detected in the products of three fishery production plants, one of which was located in Finland.

Comparison of food and patient isolates

In 2010, 17 indistinguishable PFGE profiles were found in both patients and foods (Table 1). PFGE profiles 27, 38, 44, 62, 72, 81, 96, 150, 154, 168 and 320 were found only in raw fish or fishery products. Profiles 24 and 39 were found both in fish and meat products, whereas profiles 21 and 23 were found in meat and profiles 40 and 321 in vegetables. The majority of food isolates were from foods that were likely to be consumed uncooked.

Investigation of the *Lm62* cluster

In late December 2010 and early January 2011, four cases of *Lm* serotype 1/2a PFGE profile 62 (*Lm*62) were reported in women aged >80 years. They all had been served vacuum-packed gravad salmon from a certain domestic production plant in their residential homes during Christmas. Food samples were obtained from the freezers of two residential homes and *Lm* was found in gravad rainbow trout samples in concentrations of 200 c.f.u./g and 590 000 c.f.u./g. The PFGE profiles of these strains were indistinguishable from the patient strains. However, at the same time, *Lm*62 was also detected in fish products from another producer, with levels <100 c.f.u./g.

Inspection by the municipal authorities responsible for the supervision of the plant revealed that the plant had deficiencies in its own checking and production systems. The environmental samples were investigated in-house at the non-accredited laboratory of the production plant and were analysed using the NMKL method No. 136:1990, which is an older version of the method and not specific for *Lm*; no isolates were

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Table 1. *PFGE profiles found both in patients and in foods*

PFGE *Asc*I profile	No. of patients ($n=69$)	No. of food isolates ($n=307$)	Food matrix (no. of isolates)	Food likely to be consumed uncooked
21	2	1	Raw broiler (1)	No
23	2	1	Sliced salami (1)	Yes
24	1	2	Sliced lemon marinated salmon (1)	No
			Minced meat (1)	No
27	1	1	Gravad white fish (1)	Yes
38	1	1	Baltic herring steak (1)	No
39	1	3	Cold smoked rainbow trout (1)	Yes
			Slice of ham (2)	Yes
40	1	1	Chopped onion (1)	No
44	2	1	Cold smoked salmon (1)	Yes
62	2	1	Gravad white fish (1)	Yes
72	3	3	Gravad rainbow trout fillet (1)	Yes
			Sliced cold salted rainbow trout (1)	Yes
			Rainbow trout fillet (1)	No
81	1	3	Cold smoked rainbow trout (1)	Yes
			Gravad rainbow trout (1)	Yes
			Rainbow trout fillet (1)	No
96	13	51	Rainbow trout fillet (4)	No
			Gravad rainbow trout (14)	Yes
			Ground fish (1)	No
			Cold salted rainbow trout (25)	Yes
			Rainbow trout (4)	No
			Honey marinated rainbow trout (1)	No
			Norwegian salmon (1)	No
			Halibut paté (1)	Yes
150	3	5	Rainbow trout fillet (1)	No
			Salmon fillet (1)	No
			Sandwich (1)	Yes
			Gravad rainbow trout (1)	Yes
			Cold smoked rainbow trout (1)	Yes
154	1	7	Sliced rainbow trout (4)	No
			Sliced gravad rainbow trout (2)	Yes
168	1	1	Ground rainbow trout (1)	No
320	3	1	Sliced lemon marinated salmon (1)	No
321	2	1	Salad (1)	Yes

sent to the national reference laboratory for further characterization. *Lm* had also been detected earlier in the plant during 2010, both in environmental samples and in shelf-life product samples, but the levels had been <100 c.f.u./g. Intensive cleaning measures were undertaken in December 2010 and January 2011. Several equipment parts were replaced and hygienic procedures were emphasized. The plant was requested to send the environmental samples to an accredited laboratory. As a consequence, no product samples containing *Lm* in concentrations >100 c.f.u./g at the end of shelf-life were detected after spring 2011.

Fishery production plant with persistent *Listeria* contamination of *Lm*96

In patient interviews, two cases with *Lm*96 infection reported consumption of gravad salmon produced by a certain fishery production plant. In January 2010, *Lm* concentration >100 c.f.u./g was detected in one vacuum packed cold-smoked rainbow trout produced by this plant. In addition, several other *Lm* types were also found in products produced by the same plant during 2010, three of which were identical with patient types (*Lm*168, *Lm*39, *Lm*72). A persistent *Lm*96 contamination at the production plant was

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suspected because *Lm*96 had been detected previously in products of the plant in 2008 and 2009. The municipal authorities responsible for the supervision of the plant were requested to inspect and enhance the plant's *Listeria* surveillance. Several inspections were conducted by the local authorities which showed that *Listeria* control was compromised by crossing pathways, worn-out surfaces and deficiencies in the working hygiene. In addition, *Listeria* testing did not comply with European regulations [Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs]. The shelf-life for both gravad and cold-smoked fish was shortened to 14 days. Structural changes and renovations had been carried out at the plant since 2009 and continued until spring 2010. Following intensive cleansing procedures in spring 2010, *Listeria* was not detected in the production environment. However, in 2011 *Lm*96 was again detected in the production environment, in fish products during shelf-life studies and in raw material fish, but the levels were <100 c.f.u./g.

DISCUSSION

A marked increase in the number of *Lm* infections in Finland was observed in 2010. An outbreak investigation was launched in March 2010 when *Lm*96 was isolated in patients more frequently than in previous years. In 2010, *Lm*96 was responsible for one-fifth of the reported cases. Another cluster of four cases with *Lm*62 was detected in late December/early January. Investigations revealed two fishery production plants that had problems with persistent *Listeria* strains. In addition to compromised production hygiene and structural factors, both plants had deficiencies in their internal checks for *Lm*. After intensive cleansing procedures, structural changes and renovations, *Lm* was not detected in the production environments or products of these two plants. Later, *Lm* appeared again, but the levels in the products remained below the regulatory limit of 100 c.f.u./g at the end of shelf-life. It appears that the plants were at least partly responsible for the increase of listeriosis in 2010, since the number of *Lm*96 cases fell after the control measures: *Lm*96 was found in just one patient case in 2011; however, *Lm*62 was found in seven cases in 2011.

Due to the findings of our investigation, the municipal food safety authorities were asked to ensure that the *Listeria* sampling plan that was followed at the production plants was according to the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. In addition, the authorities were urged to intensify the official supervision at the plants and food premises. Raw material fish may contain *Lm* and the production processes of cold-smoked fish and gravad fish products do not destroy it. Our experiences indicate that food production plants producing fishery products need enhanced guidance for *Listeria* control from the supervising authorities. Control measures at the plant should be precautionary and continuous and the effect of control measures should be monitored by intensified *Listeria* testing. In order to promote outbreak investigation at the national level, the municipal authorities were requested to inform national authorities on every single listeriosis case through the national registry for food and waterborne outbreaks, and genotyping of patient *Listeria* isolates was prioritized. This enabled the early identification of the *Lm*62 cluster.

The main *Listeria* transmission route to humans is through consumption of contaminated food. Since *Lm* is common in the environment, it often occurs in foods and is frequently ingested. Fish products, especially ready-to-eat products, are well known risk factors for *Lm* infections [12–14]. Fish products have also been associated with human strains of *Lm* in the neighbouring country of Sweden. Vacuum-packed gravad and cold-smoked fish was related to an outbreak in 1994/1995 [15]. In 2010, 12% of gravad and smoked fish products were shown to carry *Lm*, and 59% of the isolates had the same PFGE types as human *Lm* isolates collected during the same time period [16, 17]. *Lm* has also been isolated from dairy products, vegetables, raw meat and confectionery [1, 18–20]. Although there is no scientific consensus regarding the minimum infectious dose for *Lm*, a regulatory limit of 100 c.f.u./g in foods until the end of their shelf-life has been established based on the opinions and recommendations of the Scientific Committee on Veterinary Measures relating to Public Health in 1999 and the Scientific Committee on Food in 2000 [21].Our experience suggests that the regulatory limit might cause confusion at the production plant and among the authorities dictating control measures, since low levels of *Lm* are allowed in the products at retail level. This may affect the eradication of persistent *Lm* strains from the production environment.

In the EU, *Listeria* is infrequently detected from ready-to-eat foods in concentrations >100 c.f.u./g. Findings over this limit are most often reported

from fishery products, cheeses, meat products and sandwiches [1, 22, 23]. National surveys have repeatedly shown the occurrence of *Lm* in gravad and smoked fish products available for retail sale [11, 22, 24, 25]. For example, our national survey in 2008–2009 revealed that 2% of the tested vacuum-packed cold-smoked or gravad fishery products collected from retail grocery shops contained *Lm* with a level >100 c.f.u./g [22, 25]. Almost two-thirds of the listeriosis cases in 2010 reported consumption of vacuum-packed cold-smoked or gravad fish even those with immunosuppressive conditions or medications. THL and Evira issued two public notices during 2010 in order to remind both the authorities and the general public about which groups were most at risk from the effects of listeriosis and which food products were considered risky.

The epidemiological characteristics of the listeriosis cases were similar to those reported in previous years. The incidence increased in all age groups and was highest in those aged >70 years. Incidence of the most common listeriosis form, septic infections, increased. Five materno-fetal cases were reported in Finland in 2010; these cases are traditionally rare since between zero and three cases have been reported annually [2]. In recent years, listeriosis reporting has increased in Europe but no single reason has been identified. In Denmark, significantly more cases were reported in 2009 and the increase was seen both in septic and meningitis cases, particularly in patients in the >70 years age group [5]. In England and Wales, the number of septic cases increased in persons aged >60 years in the period 2001–2008 [8]; the increase occurred in persons with cancer or with conditions that included treatment to reduce stomach acid secretion [26].

Finding epidemiological evidence is a prerequisite to confirming the source of infection. This is challenging with listeriosis because of the long incubation period and the recall bias caused by a severe health condition or the older age of patients, as well as the high mortality rate. Questionnaires covering a wide range of foods often fail to produce reliable information. Focusing on specific food products may be useful and detection of isolates with indistinguishable PFGE profiles in patients and food could help in focusing the questionnaires on likely sources of infections. In future studies, we will markedly shorten the period for which *Listeria* exposures are enquired about; from the previous 2 months to 2 weeks for septic and meningitis cases. Recently, Goulet *et al*. [27] recommended a period of 14 days for *Listeria* exposure determination in cases with septic or meningal listeriosis. A shorter period would increase the sensitivity of the interview by reducing the recall bias.

Systematic sampling of foods from different food categories and especially during the production process is essential. It seems that the more food samples from different categories that are investigated, the more widely PFGE profiles are found to be distributed. PFGE has become the gold standard for subtyping of foodborne pathogens, and is widely used for subtyping *Lm* strains from patients and food samples. It has been shown to provide a high level of discrimination with one restriction enzyme and even higher with two enzymes [28, 29]. However, PFGE is based on a visual comparison of fragment sizes, which can be problematical [30]. The multiple-locus variable-number tandem-repeats analysis (MLVA) has been shown to have a discriminatory power comparable to PFGE and strong epidemiological concordance [31–33]. A new sequence-based method, multilocus tandem repeat sequence analysis, has been recently published [34]. With either of these methods, interpreting the data and comparing data between laboratories is easier than with PFGE. Indistinguishable PFGE profiles in isolates of human and non-human origin have been identified in several studies and many PFGE profiles have been associated with more than one food category [20, 28, 29, 35, 36]. Some PFGE profiles appear to be globally distributed. As shown by Laksanalamai *et al*. [37], using multiple typing methods, especially in outbreaks involving several *Lm* strains, could be useful. These authors used serotyping, PFGE and DNA microarray analysis to characterize the strains from an outbreak in the USA in 2011 that was associated with cantaloupe consumption. However, finding indistinguishable and temporally associated *Lm* strains in patients and food samples is not enough to identify the actual infection sources. Epidemiological investigation with adequate methods is always needed.

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DECLARATION OF INTEREST

None.

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