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Outbreak of *Salmonella* Livingstone infection in Norway and Sweden due to contaminated processed fish products

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SUMMARY

In Europe, the number of reported sporadic human cases of *Salmonella* Livingstone infection is low, and outbreaks are rare. We report the largest *S.* Livingstone outbreak described in the literature having an identified source of infection. In February 2001, an increased incidence of infection caused by *S.* Livingstone was observed in Norway and Sweden. By July 2001, 44 cases were notified in Norway and 16 in Sweden. The median age was 63 years, and 40 were women. There were three deaths, and 22 patients were hospitalized. Based on standardized questionnaires and retrospective studies of *S.* Livingstone strains in Norway and Sweden, food items with egg powder were suspected, and *S.* Livingstone was subsequently recovered from a processed fish product at the retail level. Analysis by pulsed-field gel electrophoresis documented that isolates from the fish product belonged to the same clone as the outbreak strain.

INTRODUCTION

In the past decades, human salmonellosis has become an increasing public health problem worldwide with considerable medical and economic impact [1]. In the United States, an estimated 2–4 million cases occur each year, and estimates of the annual human illness costs range from $700 million to $3.5 billion [2–4].

Modern industrial food production may result in undesired or unanticipated outcomes that pose a health hazard for consumers. Various initiatives have been launched to reinforce surveillance systems and implement preventive measures against *Salmonella* infections and other foodborne diseases. In Norway and Sweden, the epidemiological situation of *Salmonella* infections is more favourable than elsewhere in Europe, since the pandemic of salmonellosis associated with eggs never appeared in these two countries [5, 6].

The first isolation of a *Salmonella* Livingstone was made in 1951 from patients with gastroenteritis staying at a hotel in Victoria Falls [7]. Since then, *S.* Livingstone has been isolated from food animals, particularly poultry, and feed products [8, 9]. Outbreaks of *S.* Livingstone infection seem to be rare or at least under-reported. To our knowledge, less than 10 outbreaks where *S.* Livingstone was incriminated as the causative agent, have been described [10–13]. In

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these outbreaks, poultry and cheese were reported as suspected vehicles [12, 14]. The larger outbreaks were recorded in Tayside, Scotland, during 1989–1991 (71 cases, unidentified source) [10], and in several European countries in 1996 related to travel to Tunisia (26 cases) [13].

From 1995 to 2000, an average of 3–4 sporadic indigenous human cases of *S*. Livingstone infection per year were notified both in Norway [Norwegian Institute of Public Health (NIPH), unpublished data] and Sweden [15]. In February 2001, an unusual number of cases due to this serovar were reported to the NIPH [16]. At the same time, the Swedish Institute for Infectious Disease Control (SMI) also observed an increased number of cases [17]. This article describes the outbreak and the investigations that led to the identification of the source of infection.

METHODS

Surveillance

In Norway and Sweden, *Salmonella* infections are reportable to national surveillance systems for communicable diseases, and isolates from local laboratories are routinely forwarded to the national reference laboratories for verification and typing. The suspected place of infection is routinely recorded by the surveillance systems. When the increased incidence of *S*. Livingstone infections was detected in February, an alert was sent out through Enter-net, the European network for salmonellosis and enterohaemorrhagic *E. coli* infections.

Case identification

A case was defined as a resident of Norway or Sweden with culture-confirmed *S*. Livingstone infection with onset of symptoms between December 2000 to July 2001, from whom the bacterium was recovered from faeces, urine, blood or normally sterile sites. Cases reportedly infected outside Norway or Sweden were excluded.

Clinical information

Clinical data were recorded through physician notifications to the surveillance systems. For Norwegian cases, supplementary clinical information was obtained through the patients' health-care providers using a standardized questionnaire. The questionnaire covered medical history, symptoms, treatment and socioeconomic impact of the disease.

Traceback activities and environmental investigation

Pilot interviews of the first 16 case-patients identified in Norway were performed using a structured questionnaire that collected information on consumption of more than 75 food items and other exposures in the week prior to onset of illness. In Sweden the patients were interviewed with a structured questionnaire, similar to the Norwegian one. Suspected food items sampled from patients' homes and local stores were cultured for *Salmonella* using the method recommended by the Nordic Committee on Food Analysis. Animal, food and environmental *S*. Livingstone isolates routinely collected by the national *Salmonella* reference laboratories in Norway and Sweden prior to and during the outbreak were analysed and compared to human outbreak isolates. Inquiries were made in order to collect information regarding consumption of suspected food items the week before onset of symptoms for all cases.

Pulsed-field gel electrophoresis (PFGE)

S. Livingstone isolates from human cases, suspected food items and the environment were compared with epidemiologically unrelated control strains using PFGE fingerprinting after *Xba*I restriction enzyme digestion of genomic DNA, as previously described [18]. A subset of the isolates was also subjected to PFGE after overnight cleavage of embedded genomic DNA at 37 °C with *Bln*I (40 U) and *Spe*I (40 U). Electrophoresis of *Bln*I-digested DNA was performed with the procedure applied to *Xba*I digests, while DNA digested by *Spe*I was separated by electrophoresis for 22 h at 350 V and 12 °C, with pulse times from 5 to 30 s on a Beckman GeneLine II apparatus (Beckman, Fullerton, CA, USA). Unrelated control strains were provided by the Finnish and Danish institutes of public health for isolates of sporadic cases and not related to the outbreak.

Antimicrobial susceptibility

Antimicrobial susceptibility to ampicillin, ciprofloxacin, chloramphenicol, tetracycline, nalidixic acid and trimethoprim–sulphamethoxazole was tested by PDM® (Paper Disc Methods; AB Biodisk Inc., Soha, Sweden) and evaluated according to break-points

Fig. 1. Cases of *S.* Livingstone infection by week of onset, Norway ($n=44$) and Sweden ($n=16$), December 2000 to June 2001. ▩, Norwegian cases; □, Swedish cases; +, lethal.

established by the Norwegian Working Group on Antibiotic Resistance [19].

RESULTS

Characterization of the outbreak

From December 2000 to July 2001, 44 cases were notified in Norway and 16 in Sweden. The outbreak peaked in weeks 12 and 13, i.e. from 19 March to 1 April 2001 (Fig. 1). Among the 60 cases, 40 (67%) were female. The median age was 63 years (range 6 months to 94 years) (Fig. 2). Twenty-two (37%) of the cases were hospitalized [median age 73 years (range 2–89 years) and sex ratio M/F 0·35]. Three persons died in the 2 weeks following the *Salmonella* infection, giving a case-fatality ratio of 5% (3/60).

The outbreak extended from Malmö in southern Sweden to Finnmark County in northern Norway (Fig. 3). There were no prominent geographical clusters, indicating that the source was probably a product distributed all over Norway and Sweden.

No *S.* Livingstone outbreak was reported to Enter-net from other European countries.

Clinical data

Of the 60 cases reported, 54 (90%) had gastroenteritis, and among them seven (12%) had a concomitant urinary tract infection caused by the same microbe. Four cases (7%) had only symptoms of urinary tract infection, one case had an infected wound following abdominal surgery, and one case was an asymptomatic carrier.

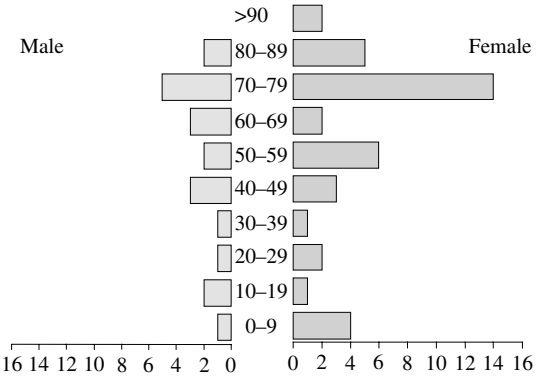

Fig. 2. Age and sex distribution of 60 cases with *S.* Livingstone infection, Norway and Sweden, December 2000 to June 2001.

More detailed clinical information was available for 33 of the Norwegian patients (11 physicians did not respond to our questionnaire). Vomiting was reported in 76% of the cases (25), fever in 55% (18). Eleven (33%) of the cases were immunocompromised because of other diseases (5 with diabetes, 4 with other diseases leading to immunodeficiency, 2 had liver dysfunction). Antibiotic treatment was prescribed to 10 patients (30%), for 5–10 days (median 8 days): ciprofloxacin (5 patients); sulphadoxine + pyrimethamine (4 patients), and metronidazole + tetracycline (1 patient). The median duration of illness reported by the physicians was 21 days (range 3 days to 7 months).

Interviews and environmental investigations

Pilot interviews of 16 Norwegian patients identified several food items consumed by at least eight of the cases. These foods were promptly purchased from

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Fig. 3. Geographical case distribution, *S.* Livingstone outbreak in Norway and Sweden, December 2000 to June 2001.

the stores identified during the interviews or collected from patients' homes and cultured for *Salmonella*. *S.* Livingstone was initially isolated from two unopened boxes (different batches) with a particular brand of 'fish gratin' produced for the Norwegian market by a factory in Sweden. No isolates were obtained from other suspected foods, including pepper and cinnamon, although routine analyses identified two *S.* Livingstone isolates in food items (egg powder and pork meat) imported to Sweden in the months prior to the outbreak. Later, the bacterium was recovered from several units of different batches of two brands of fish gratin produced by the Swedish factory and analysed by various local food control authorities in Norway. The products concerned, which contain minced fish, flour, salt and spices, egg powder (only in the product intended for the Norwegian market), is sold frozen with a shelf-life of 12–18 months and should be heated before consumption. The same factory produced a fish gratin which did not contain egg powder but mashed potato instead. The Swedish Food Administration inspected the incriminated factory and found that there were possibilities of cross contamination in the production line of fish gratin intended for the Norwegian market and that intended for the Swedish market. Thirty-eight of the 48 interviewed cases (79%) reported to have eaten one of the suspected fish gratin products the week before onset of symptoms. Six (12%) persons did not record having consumed one of these products and four (8%) did not remember.

PFGE analysis

A total of 84 *S.* Livingstone isolates were analysed by PFGE (*Xba*I). The isolates included 40 human strains from Norway ($n=28$), Sweden ($n=8$), Denmark ($n=3$) and Finland ($n=1$), isolates from fish gratin ($n=15$), other foods: pork meat ($n=1$), egg powder ($n=1$) (both imported to Sweden from Germany prior to the outbreak), environmental sources ($n=5$) and poultry ($n=5$) as well as epidemiologically unrelated control isolates ($n=17$). Thirty-five of the 36 analysed outbreak isolates from patients in Sweden and Norway had an identical PFGE fingerprint pattern. The same pattern was exhibited by all 15 isolates from different lots of two brands of fish gratin produced by the incriminated factory. Recently, a *S.* Livingstone isolate with the same PFGE fingerprint pattern as the outbreak strain was recovered from a flock of egg-laying hens in Sweden (Fig. 4).

None of the human isolates from Denmark and Finland showed this PFGE profile nor did the two isolates from the imported food items. Likewise, the PFGE fingerprints of the control strains differed from the outbreak isolates, although extensive DNA degradation made the majority of these strains untypable. PFGE after DNA cleavage with the restriction enzyme *Xba*I allowed discrimination between epidemiologically unrelated isolates. PFGE after separate application of two additional restriction enzymes (*Bln*I and *Spe*I) on a subset of 14 isolates documented outbreak isolates as belonging to a single clone genetically distinct from epidemiologically unrelated strains. While *Bln*I restriction was less discriminative than *Xba*I (Fig. 4), perfect agreement was obtained between the PFGE results generated by *Spe*I and *Xba*I. The isolates included human outbreak isolates, isolates from fish gratin and epidemiologically unrelated strains.

Interestingly, during the outbreak, seven isolates of *S.* Livingstone were recovered by routine analysis from four sewage treatment plants in Norway located in areas where no human cases were reported. The five

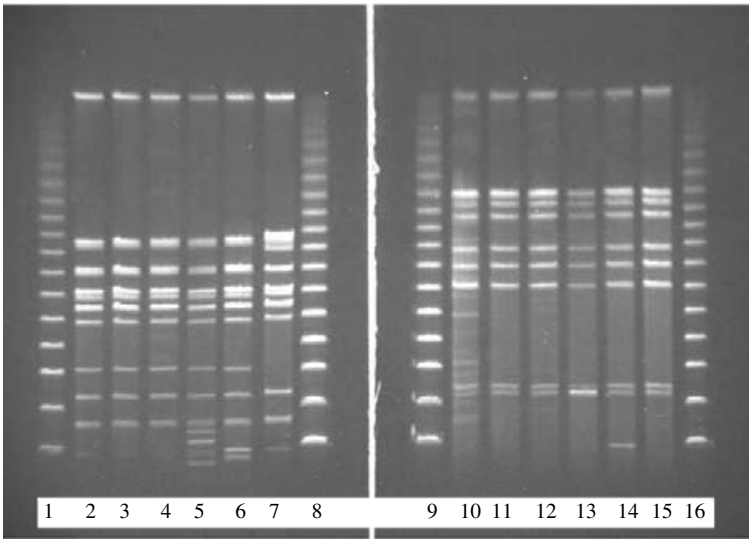

Fig. 4. PFGE profiles of *Xba*I- (lanes 2–7) and *Bln*I- (lanes 10–15) digested genomic DNA. PFGE lambda ladder as molecular marker in lanes 1, 8, 9, 16. Lanes 2, 10, Human case isolate; lanes 3, 11, poultry isolate, Sweden; lanes 4, 12, fish gratin isolate; lanes 5–7, 13–15, epidemiologically unrelated isolates.

isolates fingerprinted by PFGE represented all four plants and showed the same PFGE (*Xba*I) profile as the outbreak strain.

Antibiotic susceptibility

The 56 isolates tested (35 human, 21 environmental) were sensitive to all antibiotics tested.

Outbreak control measures

In week 13, the incriminated product was withdrawn from the market by the producer, under the supervision of the food control authorities, along with other fish products manufactured at the same production line. Public health warnings were issued in Norway and Sweden through press releases and publication in epidemiological reports [17, 20]. A notification was forwarded to other EU countries through the Rapid Alert System for Food Products on 30 May 2001. Following these public health actions, the number of reported cases fell substantially (Fig. 1).

DISCUSSION

We conclude that contaminated processed fish products, distributed in Norway and Sweden, resulted in at least 60 cases of *S.* Livingstone-related illnesses. Because of difficulty in obtaining reliable questionnaire answers from elderly persons, and the quick release of media warnings, which would have biased a study, we did not conduct an analytical epidemiological inquiry. However, 79% of the interviewed cases confirmed having eaten one of the suspected food items the week before the onset of symptoms. In addition, our finding that the fish product harboured *S.* Livingstone with the same characteristic PFGE profile as the outbreak strain and distinct from epidemiologically unrelated strains, strongly supports the conclusion that contaminated fish gratin was the vector of transmission. In PFGE analyses, three restriction enzymes were tested and evaluated and the combination of two of these (*Xba*I and *Spe*I) was well suited for discrimination. Of 60 notified human cases in Sweden and Norway, isolates from 36 cases were fingerprinted by PFGE. With one exception, all isolates had an identical PFGE (*Xba*I, *Spe*I) profile. One differed from the typical outbreak profile indicating that this case may not be part of the outbreak. This is expected bearing in mind that, based on the case definition, all indigenous human *S.* Livingstone isolates from more than half a year (December 2000 to July 2001) were collected. Along with the reported average of 3–4 human *S.* Livingstone cases in Norway each year, we would expect to find 1–2 sporadic isolates during this time period. Based on available epidemiological data and the low number of reported indigenous cases in Norway and Sweden, we have reason to believe that the 24 isolates not subjected to PFGE analyses belong to the outbreak which was caused by a single clone of *S.* Livingstone. However, it is possible that one or a few more of the putative outbreak

isolates may be from sporadic cases. Two of the six isolates from patients who did not record having consumed the incriminated fish products were typed by PFGE and both had a PFGE pattern identical to the outbreak strain. This may reflect difficulties in obtaining reliable questionnaire answers from elderly patients in a retrospectively based study or, less likely, could indicate additional products to be contaminated with the same strain. Alternatively, secondary transmission from carriers of the outbreak strain could be possible. The isolation of the *S*. Livingstone outbreak clone from four sewage treatment plants located in areas with no reported human cases probably illustrates the extensive exposure of the infecting agent to the public. Sewage treatment plants are routinely bacteriologically controlled in Norway, and isolations of *S*. Livingstone were reported only during the outbreak period (no isolation of *S*. Livingstone prior this event and none up to July 2002). This could indicate a number of healthy carriers excreting *S*. Livingstone contaminating sewage treatment plants and/or under-reporting of outbreak-related cases.

S. Livingstone has been isolated from egg-laying hens in Sweden in previous years [21]. The isolation of *S*. Livingstone from a flock of egg-laying hens where the isolate had indistinguishable PFGE patterns (*Xba*I, *Spe*I, *Bln*I) compared to the outbreak strain shows that poultry and poultry products including eggs may be a source and reservoir of this specific clone. Our investigation suggests contaminated egg powder to be the probable source of infection in this outbreak. The origin of the egg powder was unknown as both, imported and domestically produced egg powder, may be used in the production process. This information together with the finding of two *S*. Livingstone isolates in egg powder and pork meat imported to Sweden in the months prior to the outbreak, although conferring distinct PFGE patterns, illustrates that the *S*. Livingstone outbreak clone may be of foreign origin. Nevertheless, it was not possible to isolate *S*. Livingstone in egg products used in the factory.

More cases were reported in Norway than Sweden. Egg products were only used in the recipe of the fish gratin sold in Norway. The 'Norwegian fish gratin' was mixed with macaroni and a sauce containing egg powder, while the fish gratin sold in Sweden was made with mashed potatoes and an egg-free sauce. Assuming that egg powder was the source of contamination, because the production line shared common infrastructure for the production of fish gratin with or without egg powder in the recipe, it is likely that a cross contamination may have occurred. It would imply a higher contamination in fish gratin containing egg powder, and subsequently explain the higher number of cases among 'Norwegian fish gratin' consumers compared to Swedish consumers.

The wide geographical spread of human cases in Norway and Sweden was also in accordance with the widespread distribution of fish gratin products in the two countries. Because this particular product is only distributed in Norway and Sweden, the outbreak did not spread elsewhere in Europe. Indeed, the PFGE analyses of relevant isolates from the neighbouring countries Denmark and Finland indicated that the outbreak was restricted to Norway and Sweden.

The severity of the symptoms and associated urinary tract infections are not commonly described with other *Salmonella* serovar infections. Among recorded cases, the high mortality ratio in this outbreak was mainly observed among the elderly, who had associated diseases. The *Salmonella* infections appeared to cause the aggravation of another disease that was the direct cause of death (two had progressive cancer and one was immunocompromised due to another illness). The long median duration of illness was probably the consequences of the population affected, i.e. elderly persons.

The sex distribution was probably related to the high proportion of women among the elderly population and the fact that fish gratin is a traditional meal preferred by this age group.

This type of outbreak has a dramatic economic impact. Indeed, the factory was closed down for several weeks for sampling and cleaning procedures. Approximately 4 months of production of fish gratin was recalled from the market. In addition several other fish and vegetable products from the same production line were recalled and returned to the factory in Sweden for *Salmonella* control. The long median duration of illness of 21 days also implied weeks of absence from work for some cases. The direct and indirect cost for the public health authorities, health care and the producer are expected to be considerable. The factory estimated the loss to be more than 15 million Swedish Kronor (US$ 1.75 million). This underlines the severe impact of *Salmonella* infections both from a public health and economic perspective.

CONCLUSION

This outbreak is the largest *S*. Livingstone epidemic described in the literature with an identified source of

contamination. Because of the close collaboration between public health and food control authorities in Norway and Sweden, public health measures were implemented rapidly and allowed the control of this outbreak. Epidemiological investigation and genotyping methods were complementary in guiding our conclusions. *S*. Livingstone outbreaks remain a rare event, nevertheless the clinical picture observed in this investigation showed that consequences might be very severe, especially for elderly and immunocompromised individuals. The economic burden of salmonellosis infections remains a concern for public authorities.

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