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Outbreak with multi-resistant *Salmonella* Typhimurium DT104 linked to carpaccio, Denmark, 2005

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SUMMARY

We report an outbreak with *Salmonella* Typhimurium DT104 resistant to six different antibiotic classes. The outbreak occurred in Denmark in July/August 2005 and was traced to a single restaurant. In addition to patient interviews, an important tool in the investigation of this outbreak was comparison by multi-locus variable number of tandem repeat analysis (MLVA) typing of patient strains with strains from the food surveillance system. This showed that the source of the outbreak was imported beef served as carpaccio. Carpaccio, thinly sliced raw fillet of beef, has not previously been associated with outbreaks, but should be considered a high-risk food item. This outbreak was one of two in different European Union (EU) countries traced back to beef from one company in a third EU country. This underscores the importance of efficient international *Salmonella* surveillance and food-safety control systems enabling timely interventions within the EU.

INTRODUCTION

Foodborne *Salmonella* infections are a considerable cause of morbidity and most developed countries have various surveillance and intervention systems aimed at controlling the problem. In Denmark, infections in humans are mandatory notifiable by laboratories. The annual incidence of registered *Salmonella* infections has shown a downward trend in recent years from a high of 96 cases/100 000 population in 1997 to 33/100 000 in 2005 [1, 2]. This reduction is generally attributed to a series of national control and intervention programmes aimed at minimizing the incidence of *Salmonella* in domestic production animals, in particular pigs, broilers and laying hens. Intensive surveillance systems targeting different parts of the farm-to-fork chain are part of the strategy [1, 3]. Imported products, including meat products, are not part of the programmes, but are monitored for *Salmonella* by random sampling on import and, to a lesser degree, through the control of retail products.

Ready-to-eat products apart, food products sold in Denmark are generally not required to be free of

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Salmonella. One notable exception from this rule concerns multidrug-resistant *Salmonella* Typhimurium phage type DT104 (MDR-DT104), for which Denmark has special regulations [4, 5]. Food products containing MDR-DT104 (typically pork, beef or imported poultry) are not allowed to be sold, but must be destroyed or heat treated. Pig or cattle herds found with this organism are put under certain restrictions. These regulations apply to MDR-DT104 only; other types of *Salmonella* are generally tolerated. As a consequence of the special regulations concerning this organism, *Salmonella* strains isolated from food and production animals are, as a rule, always serotyped and *S*. Typhimurium isolates furthermore phage-typed and resistance tested. These surveillance data therefore also make it possible to compare, at the subtype level, the types of *Salmonella* strains found in humans and food/production animals, and are used for source attribution purposes in Denmark [1, 6]. Likewise they constitute an important resource in outbreak investigations, particularly those involving *S*. Typhimurium.

Here we describe a *S*. Typhimurium outbreak in which phage type and resistance profile of the strain from human cases were used to search for similar isolates obtained from surveillance of food and production animals. Subsequently, matching strains were further subtyped with molecular methods and the patients interviewed. The source of the outbreak was found to be imported beef carpaccio served at a restaurant.

METHODS

Several different institutions took part in the outbreak investigation. The *ad hoc* outbreak management team formed in the county where the outbreak took place involved the Regional Food Control Authority (RFCA), the county medical officer and the county clinical microbiological laboratory.

In Denmark laboratory-confirmed salmonellosis in humans is mandatory notifiable by laboratories and registered in a person-identifiable format in a database at the Statens Serum Institut (SSI). [1, 2]. Isolates are sent to the reference laboratory at the SSI and all are serotyped according to the Kauffmann–White scheme [7]. As part of the surveillance for outbreaks, the common serotype, *S*. Typhimurium, is routinely further subtyped; in 2005, when the outbreak took place, this was done by antibiogram testing, phage-typing, pulsed-field gel electrophoresis (PFGE) and multi-locus variable number of tandem repeat analysis (MLVA) typing. Antibiogram testing was performed using minimum inhibitory concentrations with the Sensititre system (Trek Diagnostic Systems, East Grinstead, UK). Phage-typing was done according to the Andersson scheme [8]. PFGE analysis was performed using the Pulse-Net standard protocol [9] and the restriction enzyme *Xba*I; data were analysed in BioNumerics (Applied Maths, Kortrijk, Belgium). MLVA typing was performed using five loci as previously described [10, 11]. MLVA is a method where variation in the number of small tandem repeats in defined loci is assessed by PCR.

The original case definition used was: cases with MLVA pattern 2-4-13-16-3. When it became clear that a restaurant outbreak was occurring, it was refined to *Confirmed cases*: patients with gastroenteritis who had eaten at Restaurant B between 1 July and 1 October and from whom the outbreak strain was isolated; *Probable cases*: the same as confirmed cases except that stool sample examination had not been performed. Interviews were conducted by telephone using a structured questionnaire.

RESULTS

The outbreak

Between mid-July and the beginning of August 2005, a cluster of five patients infected with *S*. Typhimurium of the same MLVA profile was noted at the typing centre at SSI. This fulfilled a rule-of-thumb criterion for a possible outbreak and together with the Food and Veterinary Salmonella Reference Laboratory at the Danish Institute for Food and Veterinary Research (DFVF). An outbreak investigation was initiated on 11 August. At the DFVF, a search was made for recently isolated non-human *S*. Typhimurium strains with the same phage type and resistance pattern. A total of 11 strains were found and these were typed by PFGE and MLVA. One food isolate matched the strain from the human cluster. This isolate came from imported beef from a particular Italian food producer, Company A.

In the third week of August 2005, one restaurant, Restaurant B, was mentioned in two separate notifications from physicians of cases with suspected foodborne salmonellosis. At the same time a second round of patients infected with the outbreak strain

of *S.* Typhimurium were found from the laboratory-based surveillance. Most of these patients lived in the county in which Restaurant B was located. All these patients reported eating at Restaurant B. Beef from Company A had been used at the restaurant. The beef was recalled and the outbreak stopped.

Strain characteristics

The outbreak strain was *S.* Typhimurium with MLVA pattern 2-4-13-16-3 (allele numbers at the five loci STTR9-5-6-10-3; equals the following allele sizes: 172, 246, 371, 418, and 517 base pairs). Three of the isolates had a variation in the pattern, each time in the same locus (STTR6). However, all isolates were believed to represent the same outbreak type. All strains except one had the same PFGE profile (number 205) and were phage type DT104. One isolate, which had the outbreak type MLVA pattern, differed by both PFGE and phage type. This isolate was phage-typed as 'non-typable' and had one extra band in the PFGE, compatible with a plasmid. The strains were resistant to ampicillin, chloramphenicol, tetracycline, sulphonamide, spectinomycin and streptomycin. Florfenicol resistance varied, nine strains were fully resistant, the remaining showed intermediary resistance. Three strains, however, had a different antibiogram being resistant to ampicillin and sulphonamide only, i.e. showing a *Salmonella* genomic island 1 B (SGI1-B) phenotype [12]. The genetic background for this variation is currently under investigation. Thus, to recapitulate, seven strains showed subtype variation, three by MLVA, three by antibiogram and one by PFGE and phage type. These seven strains were all isolated from patients who had eaten at the restaurant.

The isolate from beef which matched the isolates from humans had the prototype outbreak strain subtyping characteristics, i.e. it was phage type DT104, had resistance profile ACTSuSSp, PFGE profile 205 and MLVA profile 2-4-13-16-3.

Patients

Patients with the outbreak strain identified through the laboratory-based surveillance system were interviewed. A total of 31 patients reported eating at Restaurant B, an Italian-style restaurant, before onset of symptoms. All patients reported eating from an antipasti buffet; 22 patients remembered having eaten carpaccio, seven did not specifically remember or did not know what carpaccio was, and two patients were not asked this question. None of the 31 patients had travelled abroad in the week before onset of symptoms. Two patients were children aged <5 years, the remaining were aged between 11 and 57 years; 23 were female and 8 male; 11 patients were hospitalized. All patients survived, but several were ill for extended periods of time. Through the interviews, eight additional non-laboratory-confirmed cases were identified: these were persons who had eaten at the restaurant with confirmed cases and subsequently developed gastroenteritis.

An *Early Warning* (Early Warning and Response System, according to EU Council Decision 2119/98/EC) was issued on 6 September, and an Enter-net [13] *Urgent Inquiry* was issued on 12 September. A total of seven countries responded to the Enter-net inquiry. One country, Norway, reported having cases possibly connected with the outbreak, since isolates with the outbreak MLVA profile had been isolated from three Norwegian patients. Two of these were an elderly couple; the source of their infections could not be established. The third patient, when interviewed, reported having been in Denmark and eaten at Restaurant B on 25 August. The Netherlands initially responded negatively to the Enter-net inquiry, but some weeks later discovered an outbreak with the same strain of MDR-DT104 [14]. Both outbreaks, the Danish and the Dutch, were notified to the infectious community through brief, early reports in the weekly bulletin *Eurosurveillance* [15, 16] and the Danish outbreak was also reported in the national weekly epidemiological bulletin [17].

Including the Norwegian case, there were thus 32 laboratory-confirmed and eight probable cases. The patients had eaten at the restaurant over a period of 4 weeks, from 30 July to 25 August (Fig. 1). The incubation period was stipulated as the number of days between eating at the restaurant and onset of symptoms; for 31 of the confirmed and six probable cases this could be assessed (Fig. 2). One patient ate at the restaurant on two separate days. The median incubation period was 4 days. Figure 3 shows the week of stool sample submission for all Danish patients with the outbreak strain in 2005. These comprise the 31 restaurant-associated cases, preceded by the five patients that initiated the outbreak investigation and succeeded by four later cases. Before 2005 this strain was not detected in patients in Denmark during the period when PFGE (started 1 January 2003) and

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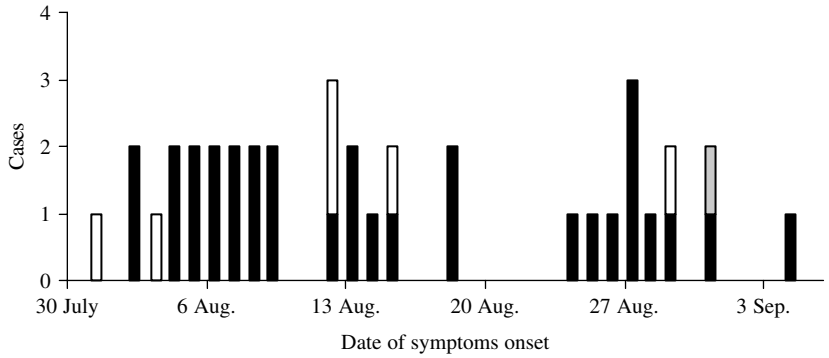

Fig. 1. Date of symptoms onset for 38 restaurant-associated cases. ▨, Norwegian; □, probable; ■, confirmed.

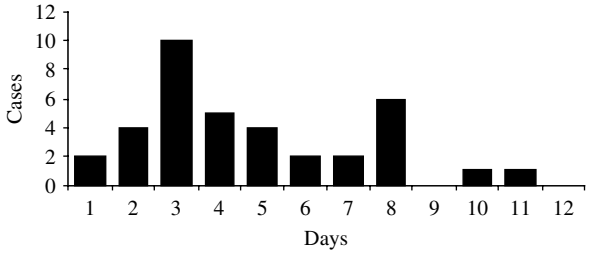

Fig. 2. The distribution of the incubation periods, in 37 cases.

MLVA (started 1 January 2004) typing was routinely performed. Moreover, *S.* Typhimurium strains of PFGE 205 or MLVA 2-4-13-16-3 from patients were not detected during 2006. Four of the first five patients (patient no. 5 could not be contacted) who initiated the molecular subtyping analysis of food and veterinary isolates had not eaten at Restaurant B, lived in different regions of the country, had not left Denmark prior to becoming ill, and did not appear to have eaten imported beef. They may have constituted a separate outbreak and the source of their infections was not discovered, since five patients were not sufficient to conduct a case-control study in the absence of a hypothesis. After the restaurant-associated outbreak ended, the outbreak strain was isolated from four other patients during 2005. Two patients were believed to have been infected while holidaying abroad (in Bulgaria and Cyprus) although one of these had in fact eaten at the restaurant in the outbreak period, but 1 month before onset of symptoms. The two remaining patients, infected towards the end of 2005 had no connection with the restaurant and the sources of their infections could not be established.

Restaurant and trace-back investigations

Restaurant B is under the control of the RFCA which – the present outbreak exempt – deemed the control programme and hygienic measures at the restaurant to be good. Carpaccio was served every day in the period of the outbreak as part of a large buffet. The carpaccio was prepared by simply slicing the partly thawed beef; no surface treatment of the beef was involved. Between 100 and 200 persons a day were estimated to have visited the restaurant and eaten from the buffet. Based on reports from patients (from the second cluster of cases), the hypothesis was formed that Restaurant B and the carpaccio were implicated. The RFCA inspected the restaurant on 26 August and found that only a small piece of beef was left. Droplets of meat juice from this piece of beef were sent for analysis and found negative for *Salmonella*. Restaurant B did not serve the beef after 25 August.

Beef from Company A was known to be imported to Denmark on two occasions by two different importers during the summer of 2005. The first batch, Batch I, was tested on 5 July as part of the governmental import control programme and found positive for MDR-DT104. Because of the Danish zero-tolerance policy on MDR-DT104, this batch of beef was not allowed for consumption and was, according to the importer, destroyed. Batch I was produced in May 2005. It was the isolate from Batch I that was found to be identical to the outbreak strain. The second batch, Batch II, consisted of 1005 kg of frozen tenderloin and was imported on 23 July. It was produced in April 2005 and was imported from Italy via The Netherlands. It was not tested for *Salmonella* in Denmark. Beef from this imported batch was sold to Restaurant B and possibly other restaurants.

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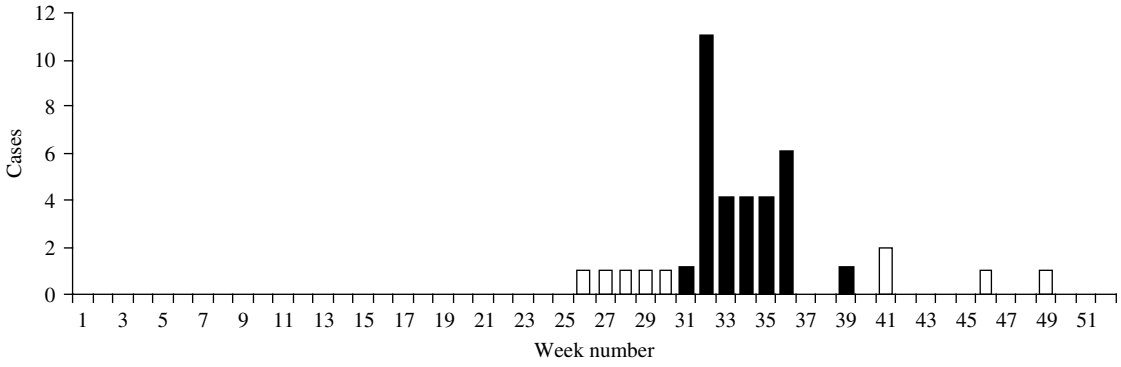

Fig. 3. Patients positive for the outbreak strain by the week the stool sample was received in the laboratory. □, Not restaurant; ■, restaurant.

At Restaurant B it was served raw as carpaccio. As a result of the outbreak investigation, the importer recalled the incriminated batch of beef from all its customers; 550 kg were returned and destroyed.

A *Rapid Alert* (Rapid Alert System for Food and Feed, according to regulation EC/178/2002) concerning Batch I was issued on 5 September (no. 2005.622) and a Rapid Alert (no. 2005.624) concerning Batch II on 6 September. The Italian Food Control Authorities responded through the Rapid Alert System that inspection of the abattoir had revealed that cross-contamination had most probably taken place in the premises that deboned and cut out meat. Cleaning and disinfection of premises and equipment were reported to have been carried out and followed up by sampling for *Salmonella*, with negative results.

We also note that a third, earlier imported batch of meat from Company A was known to be contaminated with *S*. Typhimurium MDR-DT104. A batch of pork from the company tested positive upon import into Denmark in February 2005. However, PFGE typing showed that this isolate had a profile different from the outbreak strain.

DISCUSSION

We describe a fairly large outbreak caused by multiresistant *S*. Typhimurium DT104. Infections occurred over about 4 weeks; the first registered case became infected on 30 July and the last at 25 August. Considering that the restaurant was popular and located in the centre of Denmark's second largest city, the real number of cases may well have been several times higher than the 31 Danish and one Norwegian cases found through the passive laboratory surveillance system.

The contaminated beef was prepared as carpaccio. Carpaccio is beef or veal fillet cut into very thin slices and often served with a dressing of olive oil, leaves of green salad and parmesan cheese. Because carpaccio is a raw product even a moderate contamination with *Salmonella* will result in a high risk of infection. As this outbreak shows, carpaccio, although arguably delicious, is a high-risk food. During the outbreak investigation we learned that some restaurants routinely apply a very brief surface heat treatment to the fillet before slicing it. The effect of this procedure has not been examined, but it is possible that it may reduce or eliminate the risk of infection without ruining the dish.

The outbreak was caused by Restaurant B, since all 32 patients were infected with the same strain and had eaten at the restaurant in the same period of time. All the Danish patients ate from the buffet and 22 remembered eating carpaccio. The period of time in which the beef was used for carpaccio at the restaurant (23 July at the earliest to 25 August) fits the period in which patients became infected. We believe that our investigation shows beyond reasonable doubt that beef from Company A contaminated with *S*. Typhimurium MDR-DT104 caused the outbreak.

An analytical epidemiological study was not necessary to prove that the carpaccio was involved. The patients were identified through the national laboratory notification system and the probability of obtaining by chance a large series of consecutive cases with recent visits to the same restaurant was very low. There were two further reasons for not performing such a study. First, we were unable to establish a cohort of guests at the restaurant or to sample controls

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that had eaten at the restaurant without developing symptoms. A complicating factor was the time-lag in reporting. Case finding was based on isolates from the national surveillance system undergoing further subtyping and cases were typically interviewed several weeks after onset of symptoms and eating at the restaurant. This is a limitation of molecular methods-based surveillance. Second, the available evidence based on microbiology and trace-back was considered strong enough.

Molecular subtyping methods, by comparing isolates from patients with isolates from food, played important roles in identifying the outbreak and its likely source. Initially, subtyping of isolates from the human laboratory surveillance system revealed a cluster of identical isolates suggesting an ongoing outbreak. This in turn led to a search for strains of matching serotype, phage type and resistance pattern isolated as part of the surveillance programme for *Salmonella* in production animals and food. Candidate isolates were typed by PFGE and MLVA and one isolate was found to match. The method of MLVA typing has proved valuable for subtyping *S.* Typhimurium isolates in Denmark over the last two years, performing well relative to PFGE typing with regards to cost and discriminatory power. It has efficiently distinguished subtypes of common phage types in Denmark, such as DT104 and DT12, which are not distinguishable by PFGE [18] and has been instrumental in solving one previously published outbreak [11]. However, the fact that several closely related MLVA profiles were seen in this outbreak also underscored the value of using additional subtyping methods.

MLVA also allows for comparison of results between laboratories as shown by the identification of cases in Norway as part of this outbreak. Provided that MLVA typing is performed using the same methods, results are readily communicated and compared between laboratories because an MLVA profile takes the form of a digital code. At the time of the outbreak only Denmark and Norway used the same system of MLVA typing of *S.* Typhimurium. It is possible that more patients or even other outbreaks caused by the contaminated beef would have been detected if molecular typing had been used more widely in Europe.

This outbreak allowed for a reliable estimation of incubation periods. With a median of 4 days and a range up to 11 days, these were found to be somewhat lengthy, which possibly indicates that the carpaccio was low-grade contaminated [19]. We speculate that this might mean that a sizable fraction of exposed persons did not develop symptoms. If so, this would potentially have complicated an analytical study, had one been performed. One patient, who had an incubation period of 8 days, reported having begun treatment with penicillin (for reasons other than the *Salmonella* infection) 2 days before the onset of salmonellosis. This may, therefore, represent an example of antibiotic-induced salmonellosis [20, 21]. In general multi-resistant DT104 is a particularly unwelcome type of *Salmonella* due to its increased ability to spread and the possibility of treatment failure and increased virulence [20, 22].

Most importantly, our outbreak and that from the same source of beef in The Netherlands [14] illustrate how international cooperation is becoming increasingly important. Meat products and other types of food contaminated with *Salmonella* or other pathogens frequently cross borders and may lead to outbreaks in multiple settings. Other recent examples include problems with *S.* Enteritidis associated with the import of eggs from Spain to the United Kingdom [23] and norovirus outbreaks associated with imported raspberries [24]. Food products are easily exported from country to country by virtue of the European Union (EU) open market, but outbreak investigations, trace-back and interventions directed towards food producers may become complicated when they involve several EU member states. In the US, the PulseNet system which ensures coordinated comparison of real-time PFGE typing results of *Salmonella* and other bacterial pathogens has proven to be an efficient outbreak control tool [25, 26]. Similarly, efficient flow of information about contaminated imported food products is necessary. This outbreak highlights the importance of immediately passing on information on contaminated batches through the Rapid Alert System. This gives the producing companies an opportunity to correct inappropriate procedures and at the same time also serves to warn other countries that may have imported the same types of food. In addition, good communication within countries is necessary to prevent contaminated foods from the same company being imported by different routes. During the investigation of this outbreak it became clear that the Danish Rapid Alert on Batch I was not sent in a timely manner and also that results of the import control tests performed in different parts of the country were not always efficiently distributed to other parts. Partly as a result of this,

the food control system has since been reorganized and a new central response unit established at the Danish Veterinary and Food Administration. This unit is contact point for the EU Rapid Alert System for Food and Feed and its main task is to coordinate withdrawal of contaminated foodstuffs and control measures in case of foodborne outbreaks.

In Denmark during recent years, coordinated efforts to reduce the *Salmonella* load in production animals have been successful while at the same time the consumption of imported food products has gone up. Therefore, at the population level, the distribution of the sources of infections may be changing towards an increased impact from imported foods. Work is currently ongoing to focus the Danish salmonella programmes towards maximizing the public health output. This involves plans to strengthen the current random sampling programme on *Salmonella* in imported foods with the aim of preventing import of foods considered to pose a specific risk to human health.

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DECLARATION OF INTEREST

None.

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