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Outbreak of *Salmonella* Strathcona caused by datterino tomatoes, Denmark, 2011

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SUMMARY

In September 2011, a patient cluster with a rare *Salmonella* serotype – Strathcona – was identified in Denmark. An outbreak investigation was initiated to reveal the source in order to stop the outbreak. In addition to hypothesis-generating interviews, comparable analyses of patients' household shopping receipts were conducted. A matched case-control study with 25 cases and 56 population register controls was conducted to test the findings of the hypothesis-generating investigation. In total, 43 cases of *Salmonella* Strathcona were reported in Denmark. Additionally, 28 cases were reported from Germany, Italy, Austria and Belgium. The results of the investigation in Denmark showed that 8/10 cases had bought datterino tomatoes prior to disease onset. Illness was associated with a specific supermarket chain [matched odds ratio (mOR) 16·9, 95% confidence interval (CI) 2·2–130], and having consumed elongated small tomatoes (OR 28·1, 95% CI 2·6–302). Traceback investigation showed that the tomatoes came from an Italian producer. This outbreak, linked to tomatoes, underpins the growing recognition of the broad source range of *Salmonella* and the ability of fresh produce to cause multi-country outbreaks. It is important to strengthen the international cooperation between public-health and food-safety authorities in the European Union to investigate future multi-country outbreaks in order to prevent illness from ready-to-eat produce.

Key words: Case-control study, outbreaks, *Salmonella*.

INTRODUCTION

Salmonella is the most common bacterial cause of foodborne outbreaks and the second most common bacterial cause of foodborne infections in Denmark. In 2010, 1598 cases of salmonellosis were reported, with an incidence of 28·7/100 000 inhabitants, and an estimated 45% of all cases were acquired during travel outside Denmark [1]. The most common serotype of all registered cases was *S.* Typhimurium (40%) followed by *S.* Enteritidis (24%) [1]. The food vehicles most commonly associated with *Salmonella*

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transmission worldwide are poultry, pork, beef, eggs, and seafood; although in later years, *Salmonella* outbreaks have been increasingly linked to the consumption of vegetables and fruits [2, 3].

On 22 September 2011 in week 38, three cases of *Salmonella enterica* serovar Strathcona (antigen formula 6,7:lz13,lz28:1,7) infection were identified at Statens Serum Institut (SSI), Denmark. This was the first identification of this serotype in Denmark. A literature review plus an inquiry through international networks coordinated by the European Centre for Diseases Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the U.S. Centers for Disease Control revealed that the serotype had first been isolated in 1988 in a patient in the city of Strathcona, Canada. Since then the serotype has only been reported once globally, in 2006, when isolated from a French patient returning from Cameroon with a wound infection. Between 2007 and 2010, no cases were reported to The European Surveillance System (TESSy). Similarly, there were no reports of identification of this serotype from food, feed or animals worldwide. Following the identification of the Danish cases, an outbreak investigation was initiated to reveal the source in order to stop the outbreak.

METHODS

Laboratory characterization

In Denmark, salmonellosis is a laboratory notifiable disease and cases diagnosed by clinical microbiological laboratories must be reported within a week to the SSI. Further, *Salmonella* isolates are sent to the reference laboratory at SSI for serotyping and genotyping. Serotyping was performed according to the Kauffmann–White–Le Minor scheme [4] and pulsed-field gel-electrophoresis (PFGE) according to the PulseNet protocol using the *Xba*I restriction enzyme [5]. All isolates were tested for their antimicrobial susceptibility to a panel of antimicrobials by broth microdilution (Sensititre; Trek Diagnostic Systems Ltd, UK).

Descriptive epidemiology

For the Danish investigation, a case was defined as a patient with laboratory-confirmed *S.* Strathcona infection in Denmark diagnosed during September–December 2011. International cases with the same serotype in 2011 are also described with a broader period of diagnosis from July to December 2011.

Telephone interviews with cases using a standard hypothesis-generating questionnaire were started on 26 September 2011 (week 39), while additional nationwide cases were being diagnosed. The 14-page trawling questionnaire focused on types of food consumed the week before illness onset in a variety of settings (home, takeaway, restaurant, catering, etc.) and the location of purchase of consumed foods. Open food history questions covering the 3 days prior to disease onset were also included as well as questions about other possible vehicles of infection, e.g. pets.

Comparable analyses of shopping receipts from patients' households

In addition to the interviews, comparable analyses of patients' household supermarket receipts were conducted for supermarket purchases made the 3 weeks prior to onset of symptoms. The steps of this method are shown in Fig. 1: (1) Patients were invited to participate and if they accepted, (2) provided a copy of supermarket receipts or (more often) their credit card number, date of purchase, branch of supermarket, and total amount spent on each purchase (all obtainable from their home-banking statements). (3) The data were then cleaned by removing unnecessary information and anonymized by SSI and forwarded to the supermarket chains. (4) Each supermarket chain extracted, to the degree possible, a list of purchased food items from their central computer system. (5) Data from the different households were compared at the SSI to reveal which food items had been purchased by several households. (6, 7) the Danish Veterinary and Food Administration, in correspondence with the supermarket chains, obtained further information on selected food items such as type of product, producer, country of origin, etc. Finally, (8) the results were interpreted to produce hypotheses as to the source.

Case-control study

A matched case-control study was conducted from 29 October to 1 November (week nos. 43–44) to test the findings of the hypothesis-generating investigation. Two or three controls were interviewed per case. Controls were selected from the Danish Civil Registration System [6] and matched by age (birthday), sex, and municipality of residence (to account for any difference in the distribution of retailers throughout the country and for other geographical differences). Trained interviewers, who had not

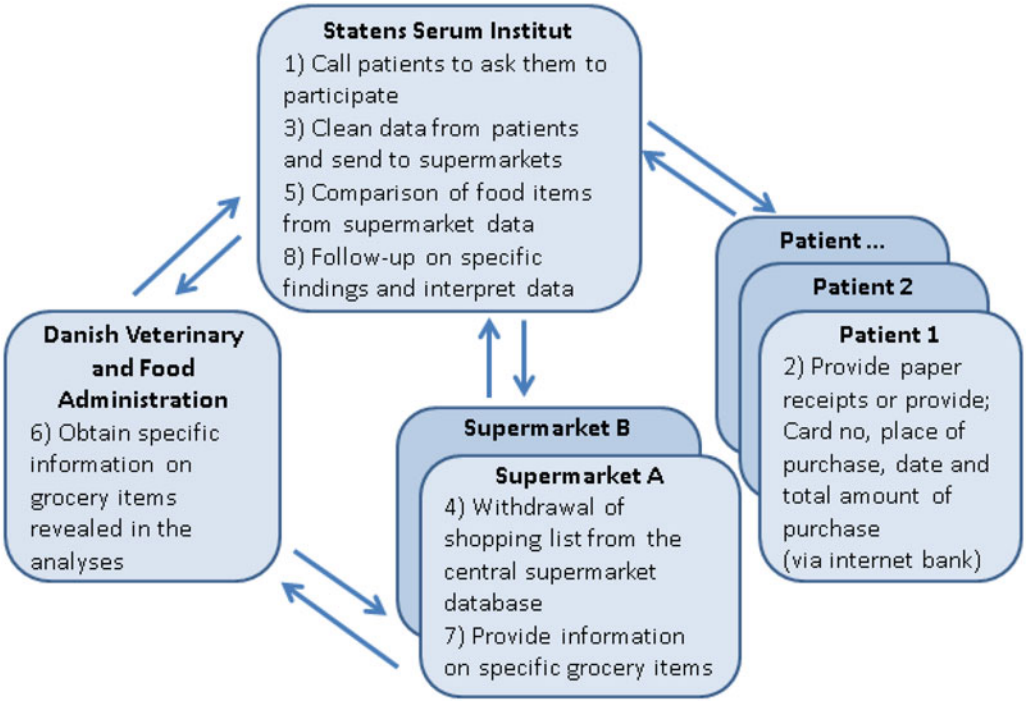

Fig 1. Data flow for comparable analyses of patients' shopping receipts, steps 1–8.

participated in the previous hypothesis-generating interviews, conducted the telephone interviews. Food items were included after an assessment of the hypotheses obtained from the trawling questionnaire results and the comparable analyses of supermarket receipts and their plausibility in relation to the descriptive epidemiology of the outbreak. Questions included consumption and location of purchase of selected cold cuts, fruits, juices, nuts and vegetables. Consumption of tomatoes was specified into two categories: large and small tomatoes and if cases confirmed consumption of small tomatoes, supplementary questions on the shape, producer and place of purchase followed.

Questionnaire data were entered into EpiData [7] and the statistical analysis conducted using SAS v. 9.1 (SAS Institute Inc., USA). Food-specific matched odds ratios (mORs) and 95% confidence intervals (CIs) were calculated. The matched analysis for the variable supermarket chain A could not run due to a lack of discordant pairs, thus the analysis was conducted recoding one control's answer from 'no' to 'yes'. The results showed that the outbreak was likely due to foods purchased from supermarket A, and therefore the analyses on individual food items were restricted to cases (almost all) and controls (about half) using this chain which meant that the matching had to be broken for this part of the analysis. Food items from the unmatched analyses restricted to supermarket A with an OR of $\geqslant 2$ were tested in multivariate analyses.

Traceback investigation

The Danish Veterinary and Food Administration conducted an intensive traceback investigation to obtain information on the origin of the source and a trace-forward investigation to explore where the implicated food vehicle was further sold. This included a notification to the European Union Rapid Alert System for Food and Feed (RASFF) on 11 November 2011 [8].

European investigation and environmental assessment

On 27 September 2011, SSI launched an urgent inquiry on the Epidemic Intelligence Information System (EPIS) for the Food and Waterborne Diseases and Zoonosis Network coordinated by the ECDC [9]. There was no comprehensive outbreak investigation done in any of the implicated countries but food histories were obtained at a regional level for some cases and compiled for additional information.

Food samples were taken in both Germany and Switzerland of later batches of datterino† tomatoes from the producer in question. Investigations

† Datterino tomatoes are small, elongated tomatoes. The shape is similar to that of dates, from which they take their name.

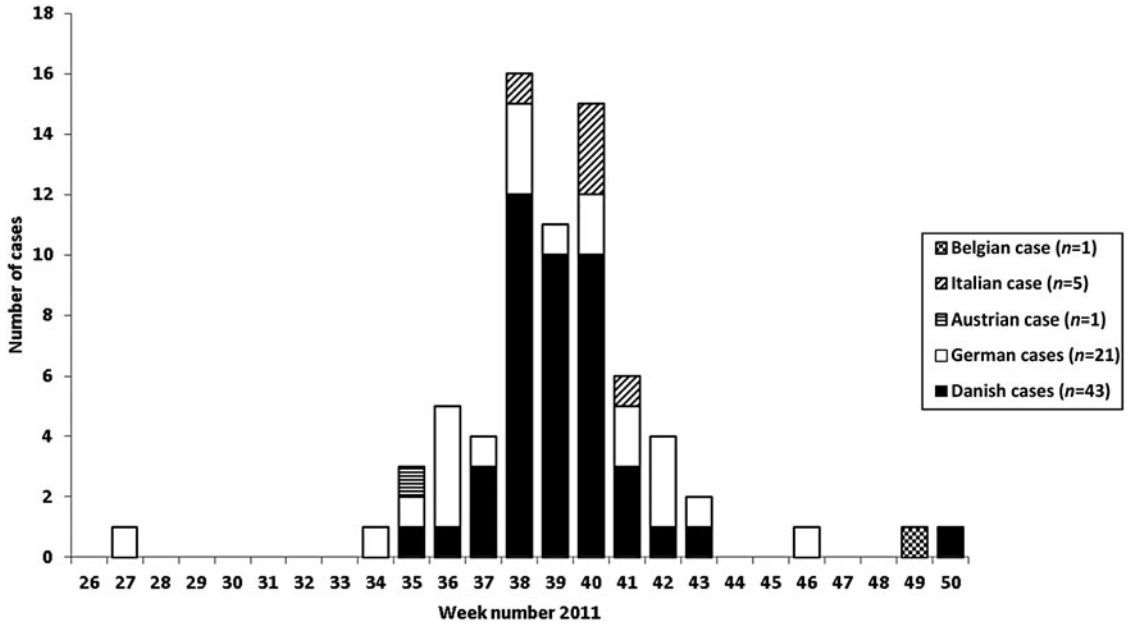

Fig 2. Week of disease onset or date of sampling, diagnosis or hospitalization for *S.* Strathcona cases in Europe, July–December 2011 ($n = 71$). (Note for the two December cases, date of sample is shown. Symptoms had been present for months.)

conducted by the Italian authorities included sampling and analyses of water used at the production site, swab samples of equipment and transport vehicles, soil samples and samples from later batches of tomatoes. The results were made available through the RASFF system on 1 December 2011.

RESULTS

Laboratory characterization

In total, 43 *S.* Strathcona cases were registered in Denmark with date of onset of illness between 4 September and 16 December 2011. For the case from December, the date of stool sample collection was used since the date of onset was uncertain as this patient had been ill for a longer period of time when diagnosed (Fig. 2). The outbreak strain was fully sensitive to all antibiotics. Genotyping of 25 isolates revealed two distinctive PFGE profiles that differed by two DNA fragment bands.

Descriptive epidemiology

The cases were aged between 6 months and 83 years (mean age 34 years) and 61% were women (Table 1). Ten (23%) cases were children aged <6 years. Patients lived throughout the country; however, some parts of Jutland and the island of Bornholm were not affected (data not shown).

All but one case reported diarrhoea (Table 1) and, in addition, the most frequent symptoms were stomach pain/nausea (80%) and fever (79%). The mean duration of self-reported illness was 12 days; however, nine cases were still ill at the time of interview and their duration is only included up to the date of interview and may therefore be underestimated. Twenty patients (47%) were hospitalized, with a mean duration of hospitalization of 5 days. *Salmonella* was isolated from the blood for six cases and from urine for one case corresponding to 16%. Six of the hospitalized patients reported underlying illness prior to the infection with *S.* Strathcona and one patient died.

Twenty-five trawling interviews were conducted (58% of the cases). These showed that the cases were infected in Denmark and no common event or specific source of infection, such as a common restaurant, was identified. The cases or their families had primarily bought food in two supermarket chains, 18 (72% of the cases) in supermarket chain A and 14 (56% of the cases) in supermarket chain B. There was an overlap of eight families who had bought food in both chains. No food item stood out as an obvious source; however, several food items were considered because they were consumed by several patients and/or seemed plausible due the time of consumption, place of

Table 1. *Characteristics of* Salmonella *Strathcona patients, Denmark, September–December 2011 (n = 43)*

Characteristic	Number of patients	Percentage
Age (years) ($n = 43$)		
0–5	10	23·3
6–20	8	18·6
21–60	14	32·6
>60	11	25·6
Sex ($n = 43$)		
Female	26	60·5
Symptoms		
Diarrhoea ($n = 41$)	40	97·6
Vomiting ($n = 35$)	16	45·7
Blood in faeces ($n = 35$)	6	17·1
Stomach pain/nausea ($n = 35$)	28	80·0
Fever ($n = 38$)	30	78·9
Duration of illness (days) ($n = 40$)*		
1–7	9	22·5
8–14	23	57·5
15–28	8	20·0
Sample type ($n = 43$)		
Faeces	36	83·7
Blood†	6	14·0
Urine	1	2·3
Hospitalization ($n = 43$)		
Yes	20	46·5
Duration of hospitalization (days) ($n = 18$)		
1–7	14	77·8
8–18	4	22·2
Deaths ($n = 43$)		
Yes	1	2·3

* Nine patients were still ill at the time of interview.
† Faeces + blood is classified as blood.

purchase, brands listed, etc. The food items further assessed were: almonds, roast beef, bananas, flax seeds, grapes, cucumbers and tomatoes.

Comparable analyses of shopping receipts from patients' households

In all, 27 patients' households were considered for the comparable analyses of shopping receipts. Of these six families (22%) were excluded, because of illness or unwillingness to participate and six households (22%) were excluded since they did not pay with credit or debit cards and had not kept their receipts. Detailed supermarket purchase data were obtained from the families of 15 cases (56%). In total data on 1185 grocery items from supermarket chains A and B were obtained and compared (Fig. 3). Information from ten families (67%), who had purchased food in supermarket chain A revealed that five food items had been purchased by more than three of the families: datterino tomatoes (8/10), cucumbers (8/10), bananas (6/10), iceberg lettuce (4/10), and apples (4/10). Information from nine families, who had purchased food in supermarket chain B revealed only one type of food – cucumbers – that were purchased by more than three families (4/9).

Case-control study

Twenty-five cases and 56 population register controls were included in the case-control study. The results of the matched analyses showed a higher odds ratio for purchasing food in supermarket chain A (mOR 16·9, 95% CI 2·2–130) than supermarket chain B (mOR 1·0, 95% CI 0·3–3·0). In total, 23 cases and 26 controls who reported shopping at supermarket chain A were included in the food item analyses. Unmatched univariate analyses showed a significantly higher odds ratio if eating iceberg lettuce, cucumber, small tomatoes, and small and elongated tomatoes (Table 2). In a multivariate analysis including elongated tomatoes, age, cucumber and iceberg lettuce, the only food item that remained significant was elongated tomatoes (OR 33, 95% CI 2·4–463).

Traceback investigation

From the information received via the RASFF network, origin of production and distribution channels of the tomatoes could be established. The producer of the datterino tomatoes was identified by the Italian authorities and the full trace records were supplied, these included the batches received by Danish supermarket chain A. Approximately 280 tonnes of tomatoes from different batches were supplied to 15 primary wholesalers and from there to secondary wholesalers in Denmark, Germany and Austria (Fig. 4). The wholesaler supplying supermarket chain A received 19·8 tonnes of tomatoes, which within Denmark were delivered in plastic containers solely to supermarket chain A. The period the tomatoes had been for sale in supermarket chain A corresponded with the period in which the cases fell ill. Trace-forward investigation showed that tomatoes from the same producer had been distributed to other European countries in the same period; Austria, Belgium, Germany, Italy, The Netherlands, UK and Switzerland.

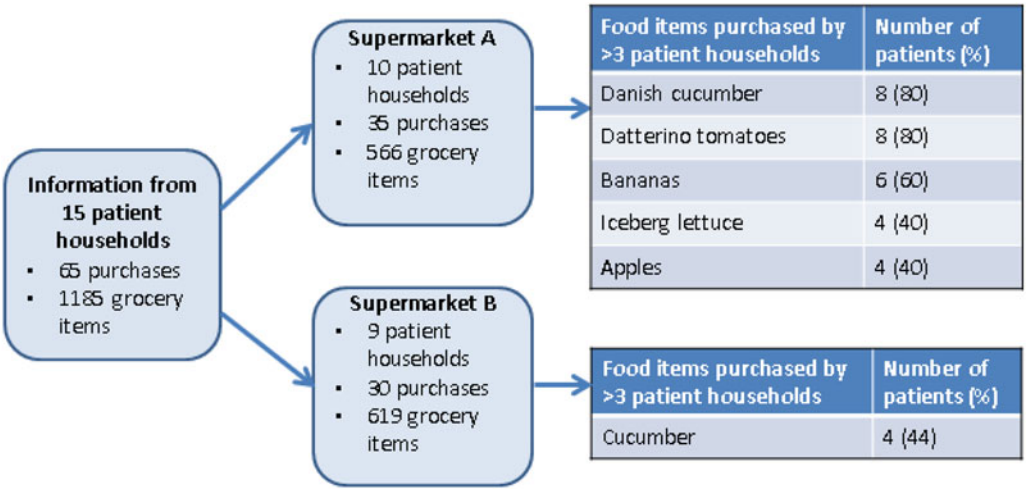

Fig 3. Outcome of comparable analyses of shopping receipts from patients' households.

Table 2. *Results of the case-control study: OR (>1·5) for food items presented, univariately adjusted for age and sex for participants purchasing food in supermarket A*

Food item	Cases who ate it/ cases included	Controls who ate it/ controls included	OR	95% CI
Cold cut: roast beef	2/23	1/26	2·4	0·2–30·9
Cold cut: fish cake	4/23	2/26	2·6	0·4–16·8
Cold cut: liver paste	16/23	15/26	1·8	0·5–6·4
Cold cut: salami	12/23	10/26	2·1	0·5–7·7
Almonds	7/23	5/26	2·0	0·5–7·6
Iceberg lettuce	15/23	7/26	8·3	1·9–36·7
Pineapple	6/23	3/26	2·9	0·6–13·5
Watermelon	9/23	6/26	2·6	0·7–10·2
Grapes	15/23	15/26	1·6	0·4–5·8
Cucumber	21/23	19/26	13·3	1·0–170
Kiwi	6/23	3/26	2·9	0·6–13·5
Large tomatoes	10/23	8/26	1·8	0·5–5·9
Small tomatoes	18/23	12/26	5·3	1·3–21·0
Small, elongated tomatoes	14/17	2/10	28·1	2·6–302

OR, Odds ratio; CI, confidence interval.

The tomatoes were no longer available on the Danish market when these results became available, so sampling and analyses of the actual batches of tomatoes suspected of causing the outbreak were not possible in Denmark and no recall of the tomatoes was necessary.

European investigation and environmental assessment

Following the urgent inquiry launched in EPIS, Germany and Austria replied within 24 h that they had seen two and one cases, respectively, and a multi-country outbreak was suspected.

In total, 28 cases were registered with laboratory-confirmed *S.* Strathcona infection from 4 July to 6 December 2011 in European countries excluding Denmark. Additionally, one German patient from January 2011 was also registered, probably being infected in Turkey. This patient was not counted as part of the outbreak. The cases were from Germany ($n = 21$ cases), Italy ($n = 5$), Austria ($n = 1$) and Belgium ($n = 1$) as shown in Figure 1. Through EPIS, 18 countries further replied that they had never registered patients with this serotype. PFGE results were available from 15 isolates from German cases and 11 of these were identical to one of the

Fig 4. Distribution of datterino tomatoes from producers in Italy to the European market, 2011.

Danish types; the remaining constituted a third PFGE profile. Two of the Italian cases and the Belgian case also shared the Danish/German PFGE profile. PFGE results were not available for the Austrian case or the remaining three Italian cases.

Three of the late-appearing German cases were specifically asked whether they had consumed datterino tomatoes sold by German supermarket chain C (Fig. 4) and confirmed they had. However, this supermarket chain was not operating in German states where 15 of the cases resided (including eight with the shared Danish/German PFGE pattern).

The Belgian case tested positive 6 December 2011; however, this was after several months of illness. She had frequently eaten tomatoes prior to disease onset. It was not possible to obtain information about tomato consumption for the cases from Italy and Austria.

In Germany, Switzerland and Italy, testing of later batches of datterino tomatoes from the producer did not reveal the presence of *Salmonella*.

The Italian authorities did not find *Salmonella* in samples taken at the production site and environment.

DISCUSSION

One batch of datterino tomatoes from a producer in Sicily was the source of this outbreak. This conclusion is based on both descriptive, analytical and food traceback arguments. In the detailed analysis of patients' purchase patterns, these tomatoes were the single most common food item purchased, demonstrated to have been bought by 80% of the patients. These tomatoes constituted 8% of total tomato sales in supermarket A and were not sold elsewhere. Moreover, the geographical distribution of cases coincided roughly with the location of supermarket A stores and cases occurred only while this batch of tomatoes were for sale in supermarket A from 18 July to 7 October. Further, the case-control study established a strong link between case status and consumption of small, elongated tomatoes sold in supermarket A. Finally, the trace-forward investigation showed that the same batch of tomatoes had not been distributed widely within Europe, but had been distributed to the EU countries where *S.* Strathcona cases were also seen in the same time period; Germany, Austria, Italy and Belgium. This also shows that the contamination occurred before the international distribution took place (in Italy). No attempts were made to isolate *S.* Strathcona from tomatoes in Denmark since tomatoes from the relevant batch were no longer available when the suspicion was formed.

At the European level, traceback of the datterino tomatoes remains incomplete. Once the suspicion about the tomatoes had been raised, food safety authorities focused on those batches still potentially on the market and were less willing to collect retrospective information. Supermarket chain C in Germany can explain only some and only comparatively late patients. Possibly, the earlier cases living in other parts of

Germany consumed datterino tomatoes from other sources left uncovered by the incomplete traceback.

It is not easy to retrieve exact information from patients' interviews on specific types of tomatoes since the patients may not easily recall package labelling or tomato type. In the present case-control study, five cases did not report consumption of small, elongated tomatoes. However, two of these had reported eating tomatoes, in the first round of interviews and a third had a receipt showing that these tomatoes had been purchased. Tomatoes have not previously been associated with outbreaks in Denmark, but they are a well-known source of infection, e.g. in the United States [10]. From other outbreak investigations, it is known that tomatoes can be contaminated at many points in the farm-to-table continuum; on farms, at packinghouses, or at fresh-cut processing facilities [10]. Tomatoes can be contaminated with animal faeces, e.g. by a polluted water source, if manure is mixed in the soil, by the hands of personnel, etc. Studies have also shown that *Salmonella* can enter the tomatoes through the roots or blossoms and lead to internal levels of fruit contamination [11–14]. Another potential source of contamination for tomatoes is the internalization of water, when warm tomatoes are exposed to cold water during processing [15, 16].

This is to our knowledge the first *S.* Strathcona outbreak reported worldwide. Because the serotype is extremely rare, the serotype alone clearly defined the outbreaks cases. Nevertheless, three different PFGE profiles were present among the case isolates. Possible explanations for this include contamination of the tomatoes from an animal reservoir where *S.* Strathcona is a normal part of the intestinal flora and different variants of the serotype could therefore be secreted, or it could be due to random reassortments or acquisitions or loss of genetic elements. The fact that this multi-country outbreak occurred with a rare serotype allowed the outbreak to be identified and investigated, which may not have been possible if the serotype had been common, because the outbreak cases may not have been distinguishable from the background cases. The successful identification of the source was probably enhanced by the fact that the tomato type involved was distinct and specific. The full extent of the outbreak remains unknown since the cases observed were only those who were laboratory-confirmed and we did not capture all cases, e.g. those that did not seek healthcare, were not tested in the healthcare system or where isolates were not further serotyped.

The information we have from the cases in this outbreak might indicate that the patients were more severely affected by the infection than the general manifestation seen for *Salmonella* in Denmark. In a review of a Danish cohort of 3328 cases from 1991 to 1999 the hospitalization rate among outbreak cases was 29% compared to 47% in this outbreak [17]. Fisker et *al.* found that 4% of the *Salmonella* cases in their cohort had extraintestinal *Salmonella* defined as *Salmonella* cultured from patients' blood samples and/or other extraintestinal infectious focus [17]. In this outbreak, *Salmonella* was isolated from the blood or urine for seven (16%) cases. The severe manifestation in this outbreak could be due to a high dose of *Salmonella* in the tomatoes or it could be that this serotype is more virulent, but since information on the level of contamination in the tomatoes is not available this remains unknown.

Conducting comparable analyses of shopping receipts from patients' households has previously been successful during an outbreak investigation in Denmark [18] and we would likely not have identified the source without the use of this method. The fact that the product was sold in one supermarket chain only and appeared in the receipts under a specific product name and product number helped make the method a success. The method has also been used during another *Salmonella* outbreak investigation, where it was not possible to reveal any hypotheses of the source despite the inclusion of more than 50 cases in the investigation [19]. A drawback of this method is that it is very time consuming, due to the many steps in the process. To get the full picture of what a patient may have consumed data is needed from all members of the household who buy food. Another limitation is that the data collected from the patients on credit card number, time and place of purchase and amount is considered by some persons as very sensitive data, which they are not comfortable in sharing. It is also a limitation that patients with purchases made in cash with no paper receipt kept are excluded from the investigation. In this outbreak four patients were excluded due to this limitation.

Epidemiological information was not available for all *S.* Strathcona patients outside of Denmark, but since the datterino tomatoes from the Italian producer were sold in supermarkets in these countries, it seems highly likely that they were ill from the same source. No immediate control measures were put in place in this outbreak since the source was revealed at the time, when the batches of tomatoes were no longer

on the market and due to the fact that *Salmonella* was not detected in the samples taken from the production site, equipment and successive batches. However, long-term preventive measures should be considered in order to ensure, that similar outbreaks will not occur. Since tomatoes are considered a ready-to-eat product and there is a risk that *Salmonella* is present inside the tomatoes, the control measures cannot focus alone on consumer behavior, e.g. rinsing the tomatoes, but need to also target the management of the production, e.g. by applying good agricultural practice (GAP) or hazard analyses critical control points (HACCP) principles and ensuring that the critical control points within the production chain are correctly assessed and the procedures complied with to avoid and control contamination of the tomatoes before they reach the consumers.

The ECDC-coordinated platform EPIS and the EC-coordinated RASFF were useful communication channels to ensure early identification of a multi-country outbreak. However, the communication could have been improved by ensuring a two-way communication as suggested by Ammon & Tauxe to also share the lessons learned from the outbreak at all levels from farm to table in order to ensure long-term prevention [20].

A good example of successful communication in the aftermath of an outbreak took place in 2010 and 2011 when Denmark experienced several norovirus outbreaks that could be linked to raspberries from the same Serbian producer. These findings led to a successful international investigation with visits and consultations between Denmark and Serbia following implementation of preventive measures at authority level and at food-business operator level [21]. It is important to bear in mind that prevention is of mutual interest since the food producers have a main interest in keeping their products safe in order to maintain their trading market.

In conclusion, this outbreak associated with tomatoes underpins the growing recognition of the broad source range of *Salmonella* and the ability of fresh produce to cause multi-country outbreaks. EPIS and RASFF are important communication channels to ensure early identification of multi-country outbreaks and outbreak investigation. It is important to strengthen the international cooperation between public-health and food-safety authorities in the European Union to investigate future multi-country outbreaks in order to prevent illness from ready-to-eat produce.

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DECLARATION OF INTEREST

None.

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