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Salmonella enterica serovar Agona European outbreak associated with a food company

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SUMMARY

We investigated an international outbreak of *Salmonella* Agona with a distinct PFGE pattern associated with an Irish Food company (company X) producing pre-cooked meat products sold in various food outlet chains in Europe. The outbreak was first detected in Ireland. We undertook national and international case-finding, food traceback and microbiological investigation of human, food and environmental samples. We undertook a matched case-control study on Irish cases. In total, 163 cases in seven European countries were laboratory-confirmed. Consumption of food from food outlet chains supplied by company X was significantly associated with being a confirmed case (mOR 18·3, 95% CI 2·2–149·2) in the case-control study. The outbreak strain was isolated from the company's pre-cooked meat products and production premises. Sufficient evidence was gathered to infer the vehicles of infection and sources of the outbreak and to justify the control measures taken, which were plant closure and food recall.

Key words: Foodborne infections, *Salmonella*.

INTRODUCTION

On 15 July 2008, the National Salmonella Reference Laboratory (NSRL) in Ireland reported to Ireland's Health Protection Surveillance Centre (HPSC) six *Salmonella enterica* serovar Agona (*S.* Agona) isolates. The isolates were from cases with dates of illness onset between 23 June and 7 July 2008. Prior to June 2008, the NSRL had identified fewer than ten *S.* Agona isolates per year (NSRL, unpublished data). Given that Ireland and the UK share many food producers, information on the increase was passed to agencies in the UK. As a result of the information from Ireland together with the identification and

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designation of a new phage-type in England & Wales, cases of *S.* Agona in England & Wales, and Northern Ireland were reviewed prospectively and retrospectively. The Health Protection Agency (HPA) identified 32 *S.* Agona isolates designated with a new phage-type PT39, a small cluster having been identified as occurring in April 2008. This cluster was not above expected levels for that period, and hence no investigation was initiated. The Scottish Salmonella Reference Laboratory reported that they had also identified 15 *S.* Agona isolates since 25 June 2008. Pulsed-field gel electrophoresis (PFGE) had been performed on 12 of these isolates: 11 shared the same distinct pattern (designated SAGOXB.0066 by PulseNet Europe) seen in the six cases from Ireland. The HPSC declared an international outbreak on 18 July 2008.

METHODS

Early investigation

In keeping with accepted practice in Europe, as Ireland had initiated the international inquiry, the HPSC convened and chaired the international outbreak control team (IOCT) with representatives of public health and food safety agencies of Ireland, England, Wales, Northern Ireland and Scotland.

A confirmed case was defined as a person from whom *S.* Agona with the PFGE profile SAGOXB.0066 had been isolated since 1 January 2008. *Salmonella* reference laboratories in Ireland and the UK performed national case-finding. HPSC issued international alerts on 23 July 2008 via the European Union Early Warning and Response System (EWRS) [1], and the Food and Waterborne Disease network (European Centre for Disease Prevention and Control; ECDC) [2], and on 14 August 2008 in *Eurosurveillance Weekly* [3]. National public health institutes reported confirmed cases to HPSC.

Following detection of the cluster of cases in Ireland, the NSRL reviewed its records and identified a *S.* Agona isolate received from a private laboratory in May 2008. PFGE confirmed the isolate as SAGOXB.0066. The source of the isolate was not specified by the referring laboratory and was not immediately available to the IOCT. However, on 25 July 2008, the Irish Department of Agriculture, Fisheries and Food were able to confirm to the IOCT that the source of this isolate was an Irish food production company (company X). The isolate was the result of a cooker failure leading to undercooked bacon on one production line in company X [thermal zone 1 (TZ1)], on 24 April 2008. The cooker failure was identified at the time by company X. All the product was quarantined and sampled, and all production was suspended. Deep cleaning was subsequently performed. Production did not recommence until results from environmental swabs indicated *Salmonella* was not detected. Company X was a major exporter of pre-cooked meats used as sandwich fillers and pizzas toppings (~800 tonnes/week), that were distributed across Europe. Company X's primary customers included two main food outlet chains (A, B) and a pizza chain (pizza A). Intermediate distributors supplied a number of other customers (among them food outlet chain C).

Given the evidence available, the working hypothesis was that consumption of food from a food outlet supplied by company X was associated with being a case. On 31 July 2008, the HPA informed the IOCT that the outbreak strain had been isolated from a pre-cooked beef product originating from company X sampled from a chain A outlet in Northern Ireland on 3 July 2008. The implicated production line (TZ1) in company X (and as a precaution, adjacent zones TZ2 and TZ3), was consequently closed on 1 August 2008. Pre-cooked meat products from company X's plant that were similar to the product from which the outbreak strain was detected were recalled from the market.

Hypothesis generation and epidemiological studies

A review of 17 trawling interviews with early cases demonstrated that eating sandwiches bought in food outlet chains was reported by three-quarters of respondents, but no evidence pointed towards any particular food outlet chain or food item. Consequently, the IOCT prepared a questionnaire to collect socio-demographic characteristics, clinical information on gastrointestinal symptoms, and to assess exposure to a variety of food outlet chains in Ireland and the UK. The questionnaire aimed to determine if any link existed between cases and food outlet chains supplied by company X. The questionnaire sought information on all possible food outlet chains in any particular country. Cases in the UK with a date of symptom onset from 1 June 2008 who had not yet been interviewed received the descriptive questionnaire. All Irish cases were re-interviewed.

In addition, a case-control study was undertaken in Ireland between the 13 and 19 August 2008 with cases

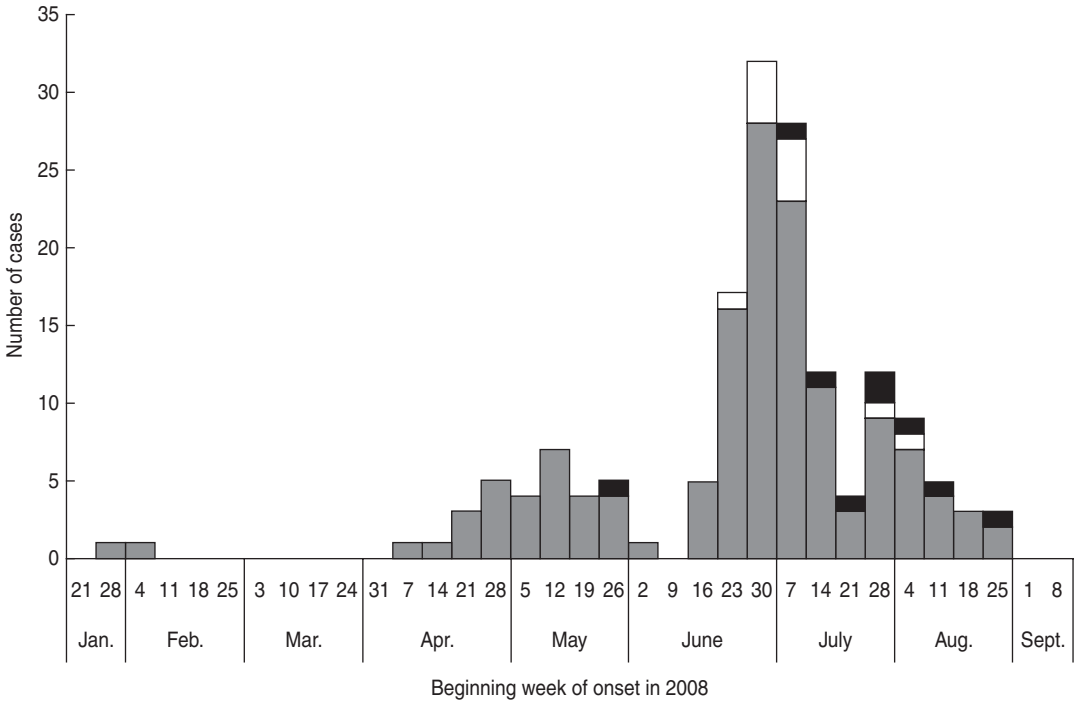

Fig. 1. Confirmed cases of *Salmonella* Agona, 2008. □, Ireland; ▒, UK; ■, other.

and controls matched on age, sex and area of residence to test the hypothesis that cases were more likely than controls to have eaten in food outlet chains supplied by company X. Three potential controls per case were identified and contacted using random digit dialling. A total of 34 controls were enrolled. Controls were questioned about the same 3-day exposure period (as applied to their equivalent cases) 2 weeks before the day of the interview (prior to the initial public report of the outbreak).

Data analysis

Statistical analysis was performed using Stata version 9.2 (Stata Corporation, USA). It included basic descriptive statistics, matched odd ratios (mORs) relating to food outlet chains and specific food items and their 95% confidence intervals (95% CIs), calculated using conditional logistic regression. A two-tailed P value $<0{\cdot}05$ was considered significant.

Environmental investigation

The Department of Agriculture in Ireland investigated company X. The Food Safety Authority of Ireland and the Food Standard Agency in the UK led the environmental investigation. Environmental health officers inspected a sample of food outlets known to be supplied by the company and those outlets mentioned by the cases. They collected samples for culture from opened and unopened batches of finished products and from environmental surfaces in inspected outlets. Stool specimens from workers in company X were cultured for *Salmonella*. Food-safety management procedures in company X were reviewed.

Laboratory investigation: molecular typing

Standard methods for PFGE (PulseNet protocol using restriction endonuclease *Xba*I) were used to subtype *S.* Agona isolates. We compared PFGE profiles using Bionumerics software (Applied Maths, Belgium) and compared profiles using algorithms available within the PulseNet Europe database. We produced dendrograms using the unweighted pair-group method using arithmetic means.

RESULTS

Case-finding

A total of 163 confirmed cases were identified (Fig. 1) from Ireland ($n=11$), UK ($n=143$) [England ($n=96$), Wales ($n=11$), Northern Ireland ($n=2$), Scotland ($n=34$)], France ($n=3$), Luxembourg ($n=2$), Sweden ($n=2$), Finland ($n=1$) and Austria ($n=1$). Onset dates ranged from January to September 2008. The

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Table 1. *Total number of cases, cases theoretically eligible* for descriptive study, and response rate by country,* Salmonella *Agona outbreak, Ireland and UK, summer 2008*

	Ireland	England	Scotland	Wales	Northern Ireland	Other countries	Total
Total number of outbreak cases	11	96	34	11	2	9	163
Eligible*	11	61	29	7	1	0	109
Questionnaire completed	11	29	9	6	1	0	56
Response rate (%)	100	47·5	31	85·7	100	0	51·4

* Cases with date of onset from 1 June 2008.

epidemic curve showed two epidemic peaks in spring and summer 2008 suggesting that food products contaminated with an indistinguishable *S.* Agona may have been on the market for a period in spring and again in summer of 2008. The median age of cases was 27 years (range 3 months to 87 years), 92 cases were male (57%). Twenty-five cases (15%) were hospitalized. Two elderly patients infected with *S.* Agona SAGOXB.0066 and who met the outbreak case definition died.

Descriptive study

Data were collected from 56 eligible cases (51·4%) as part of the descriptive study (Table 1). Symptoms included diarrhoea (98%), abdominal pain (93%), nausea (73%), fever (54%), vomiting (41%) and blood in stool (39%). Responders and non-responders did not differ in age and sex distribution.

Twenty-nine cases (51%) recalled eating food from four food outlet chains directly or indirectly supplied with products from company X. Food outlet chain A and chain B sold sandwiches: chain A operated in Ireland and the UK while chain B only operated in Ireland. Twenty-two cases ate at chain A, 19 of whom consumed beef sandwiches, containing the same product as that found to be contaminated in Northern Ireland on 3 July. The remaining three cases purchased sandwiches containing cooked bacon, cooked pepperoni, and vegetables, respectively. The pepperoni was from company X, but not produced on TZ1. Four Irish cases (36%) reported eating sandwiches from chain B; three sandwiches purchased in chain B contained chicken, and one of these also contained ham. The last sandwich was made with hot bacon. Chain B received a range of products from company X including bacon prepared on TZ1. One chain B outlet had contaminated pre-cooked bacon on the premises. This bacon product was the same type as that eaten by the case in chain A.

Chain C sold sandwiches and operated in both Ireland and the UK. It received products from company X through a number of intermediaries. One Irish case reported consumption of a ham sandwich from a chain C outlet.

Pizza chain A operated in both Ireland and the UK. Two Welsh cases ate products that did not contain toppings from TZ1. Cross-contamination may have occurred on premises.

No other common exposure was identified in cases, and no other chain was known to be supplied by company X.

Irish analytical study

All 11 Irish cases were included in the study with 34 controls matched on age (28·2 years *vs.* 29·0 years for controls, $P<0·82$), sex (45% of cases *vs.* 47% of controls were female, $P<0·96$) and area of residence. None of the controls had diarrhoea during the 2 months prior to interview. None of them had travelled abroad during the matched cases' incubation period.

Cases were more likely than controls to have eaten sandwiches purchased in food outlet chains (90·9% *vs.* 38·2%, mOR 15·8, 95% CI 1·9–132·6); eight of 11 cases and four of 34 controls ate in chain outlets supplied by company X. Among all the food outlet chain settings where Irish cases had purchased sandwiches, chain A and chain B were the only two chains significantly associated with being a case (36·4% *vs.* 5·9%, mOR 10·2, 95% CI 1·1–93·0). Exposure to food outlet chains in Ireland supplied by company X (mOR 18·3, 95% CI 2·2–149·2) and sandwiches prepared with pre-cooked meat produced by company X (mOR 15·8, 95% CI 1·9–132·6) were significantly associated with illness (Table 2). The case-control study failed to identify any association with chain C. No control had been exposed to chain C, therefore the association could not be tested.

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Table 2. *Association between illness and sandwiches bought in food outlet chains supplied by company X in Irish cases,* Salmonella *Agona outbreak, Ireland, summer 2008*

	Cases ($n=11$)		Controls ($n=34$)		
Food and chain exposure	*n*	(%)	*n*	(%)	mOR (95% CI)
Consumption of sandwiches from food outlet chains	10	(90·9)	13	(38·2)	15·8 (1·9–132·6)
Chain A or chain B	8	(72·7)	4	(11·8)	18·3 (2·0–149·2)
Chain A	4	(36·4)	2	(5·9)	10·2 (1·1–93·0)
Chain B	4	(36·4)	2	(5·9)	10·2 (1·1–93·0)
Chain D	1	(9·1)	1	(2·9)	3 (0·2–48·0)

mOR, Matched odds ratio matched on age, sex and area of residence; CI, confidence interval.

Environmental investigation

The outbreak environmental investigation led to the isolation of the outbreak strain in pre-cooked beef samples from opened and unopened packs in five chain A outlets based in Ireland, Wales and Northern Ireland. Some of these products were supplied frozen with a shelf life of 1 year. A pre-cooked bacon sample taken in a chain B outlet in Ireland also tested positive for the outbreak strain. The food that tested positive was produced on TZ1.

In addition, two outbreak environmental samples taken on 30 July 2008 from the low-risk areas in TZ1 and TZ2 tested positive for the outbreak strain. Several meat samples (pre-cooked bacon, raw bacon and beef) taken in company X premises during June 2008 tested positive for *Salmonella* and were subsequently found to be positive for the outbreak strain in the first week of August. *Salmonella* was not isolated from faecal samples from company X staff.

Laboratory investigation: molecular typing

All *S.* Agona strains isolated from patients, company X meat products, and from environmental sites in TZ1 and TZ2 had the PFGE pattern SAGOXB.0066 and were indistinguishable, or showed one band difference. All isolates were fully susceptible to antimicrobial agents tested.

Control measures taken

Following identification on 31 July 2008 of the outbreak strain from a pre-cooked beef product from a chain A outlet in Northern Ireland sampled on 3 July 2008, batches produced at the same time were recalled and production on TZ1, TZ2 and TZ3 stopped. Very large quantities of different products produced on TZ1 were progressively withdrawn from the market over a number of weeks as increasing epidemiological, microbiological and environmental evidence accumulated implicating other products on TZ1. Extensive decontamination using hydrogen peroxide was performed in the plant. All production staff was retrained; and ovens externally re-validated. TZ1 was decommissioned. A number of alerts were sent out through the Rapid Alert System for Food and Feed (RASFF).

Distribution pathways

Forty-six pre-cooked meat products had been produced on TZ1 since May 2008; 36 were known to have been distributed across Europe and one to Kuwait. Company X kept records on product distribution to their immediate customers. However, the distribution pathways included a number of intermediaries making the distribution supply highly complex and complete information could not be ascertained.

DISCUSSION

We investigated a large international outbreak identified initially by laboratory-based surveillance as a temporal and geographical cluster of *S.* Agona of an uncommon subtype. The large number of laboratory-confirmed cases probably represents a minority of actual cases in this outbreak [4–6]. Epidemiological and microbiological investigations identified company X as the most likely, though not necessarily only, source of the outbreak. More than half of interviewed cases reported having eaten sandwiches/pizza purchased in food outlet chains supplied with pre-cooked meat products supplied by company X. In the

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case-control study, exposures to food outlet chains supplied by company X were statistically significantly associated with illness. Strains isolated from the cases, meat produced by company X, and the environmental sites in the plant all exhibited the PFGE pattern SAGOXB.0066. The closure of the plant and recall of the implicated meat ensured that contaminated products were removed from the market and ended the outbreak. However, one confirmed case in Ireland and 11 in the UK appeared over the subsequent 6 months. Consumption of residual contaminated products (the implicated beef product was supplied frozen with a 1 year assigned shelf life) that had remained on the market could account for these cases; however, this hypothesis could not be confirmed.

A review of data on *S*. Agona at the NSRL in Ireland indicated that *S*. Agona SAGOXB.0066 had been detected in Irish poultry and pigs before the outbreak (2005 and 2006). It is possible that this strain may be established in a range of animal reservoirs and that therefore one or more of the cases that appeared belatedly may represent sporadic cases of infection unrelated to company X. A number of other *S*. Agona PFGE types have also been detected previously in Ireland including a type associated with a small local outbreak. However, the strain responsible for the 2005 outbreak had a different PFGE pattern from this outbreak strain, it had a different antimicrobial sensitivity profile being resistant to ampicillin and streptomycin (the more recent *S*. Agona outbreak strain was fully sensitive). It is interesting to speculate if there are specific properties of this strain of *S*. Agona that confer the potential to be associated with an outbreak such as heightened potential to cause disease and/or to persist in the food-producing environment. Studies comparing the ability of the strain to form and persist in biofilms relative to other strains of *S*. Agona and *S*. Typhimurium are underway. However, to date no specific attributes that explain the association of the strain with the outbreak have been identified.

Although we identified the most likely vehicle and source of infection, the cause of contamination within the plant remains unknown. A documented episode of contamination of pre-cooked meat with an indistinguishable *S*. Agona strain in April 2008 was attributed to a failure of the TZ1 oven. The batch of product recognized as contaminated at that time was not placed on the market and measures to resolve the problem including cleaning and decontamination of the plant were taken. A cluster of cases in the UK was temporally associated with the April contamination incident; two of the early English cases with onset in late April ate products from food outlet chains known to be supplied by company X.

It could be speculated that the episode in April may also have led to dissemination of *S*. Agona in the post-cooking part of the plant and that the strain persisted in a specific niche environment on the 'cooked-side' despite the extensive cleaning and decontamination.

Previously, *S*. Agona outbreaks have been associated with various vehicles [7–13]. The pre-cooked meats identified as the vehicle of infection in this outbreak were distributed internationally to a number of food outlet chains, directly or through intermediaries. This outbreak illustrates the complexity of food-distribution systems and how different food items and numerous brands can have a single source [14, 15]. This can significantly complicate epidemiological investigation and implementation of product withdrawal or recall. In the UK, the distribution network proved to be even more complex than that in Ireland and the linkage more difficult to make. The results of the descriptive study in the UK, the Irish case-control study and the overall microbiological evidence were sufficient to take appropriate control measures and therefore no case-control study was performed in the UK.

Once the international dimension of this outbreak was recognized, collaboration between countries worked well. This is a critical issue in managing extensive international food outbreaks. In Europe, the single market permits free movement of goods between member states. This allows for widespread distribution of food products [16–20] and can make the recognition of international outbreaks difficult. Modern communication tools used in a timely manner [14, 21, 22] are helpful in controlling such outbreaks. Formal exchange including European Union's EWRS [1], the Food and Water Disease network (ECDC) [1], rapid communication in *Eurosurveillance Weekly* [3] and the RASFF of the European Union [23] were used to communicate the outbreak, to find cases and to take appropriate control measures. Molecular subtyping methods comparing isolates from cases, food and the environment played a crucial role in the detection of the outbreak and in determining the extent and the outbreak source [24]. Cases were quickly confirmed by exchanging molecular data through the PulseNet Europe database [25]. This underlines the value of rapid subtyping

by reference laboratories and subsequent reporting of unusual/novel pathogens to PulseNet Europe.

Laboratory-based typing also played an important role in rapidly linking the outbreak to a specific source. The laboratory that had isolated the *S.* Agona in April had voluntarily submitted the isolate to the NSRL for typing. Although limited information was available to the NSRL regarding the source of this isolate, a link was suspected when human cases were identified. This suspicion was reinforced when PFGE typing was performed and it was possible to link back to the food business through the laboratory. The definitive typing techniques used during this outbreak helped ensure it was rapidly identified, investigated, and brought effectively under control. Without such typing this outbreak is unlikely to have been detected, and certainly not at such an early stage.

The distribution of meat products was extensive and complex making tracing of implicated products difficult. Despite the requirement of the European General Food Law Regulation which makes mandatory the ability for the implicated food to be traced, it was challenging to obtain comprehensive distribution information. Company X met its legal obligations under Irish food law in that it was able to identify and had kept records of food distributors and outlets to which it had supplied food.

Nevertheless, the IOCT established that food components produced by company X were used in final products that the original manufacturer was unaware of and had not intended, thus making epidemiological investigation more complicated. This resulted in a lengthy investigation and a longer time elapsing before the application of control measures than would otherwise have been the case. The issues raised by this outbreak reinforce the importance of having in place rapid and effective traceback systems to ensure that the exposure of cases can be identified and that recall and other interventions can be applied rapidly. Given the extent of this outbreak and the potential for widespread distribution of such meat products, it is important that barriers to the ability of authorities to implement control should be minimized.

A learning point from this outbreak is that the capacity to make links between human illness and food products should be strengthened. Detection of pathogens such as *Salmonella* during routine sampling of products that have previously received a treatment that is intended to kill the pathogen (such as isolation of *Salmonella* from cooked meat) indicates a breakdown in hygiene. It is important to ensure that failure of cooking in a continuous-line cooker should not result in undercooked (raw) material entering a high-risk (cooked product) zone in a food plant.

Pathogens isolated from raw materials, the environment and cooked products should be serotyped and the results subject to regular review. This will enable detections of patterns of particular serovars suggestive of on-site colonization, facilitating timely corrective action. Such isolates should be stored for 2 years, have antibiotic sensitivity documented and be definitively typed using molecular techniques in an outbreak setting.

Recognition and management of international outbreaks is a major challenge given the complexity of modern food production and distribution systems. Formal structures for rapid exchange of inquiries and information (such as provided by European Commission and the ECDC in Europe) are most valuable; however, the cultivation of professional networks through a process of periodic meetings to exchange experience are a vital element in ensuring that information exchange is effective. In Europe such a professional network of epidemiologists and microbiologists engaged in the control of foodborne infection has been developed over a period of more than 15 years through the SalmNet and EnterNet projects and more recently the European Food and Waterborne Disease Network supported by ECDC. Standardized subtyping of isolates can assist in confirming that isolated cases or small numbers of cases in additional countries may be linked to the outbreak even when it may appear initially that the product of concern is not distributed in those countries.

The possibility of other sources of this PGFE pattern of *S.* Agona contributing to some of the cases could not be ruled out. There were a small number of other possibilities; there may be a totally independent source for this infection or the supplier of the initial contaminated material to company X may also have supplied other companies with contaminated source material. These were considered but the hypothesis that company X was the most likely source remained. Cases had appeared at about the same time as the cooker failure, and when control measures were applied to product produced by company X, the outbreak resolved.

The outbreak control team did not identify any other potential sources at the time of the outbreak investigation but subsequent to the completion of the investigation information on raw chicken

contaminated with the same strain of *S.* Agona in an Irish source became available. Contamination of raw chicken is common but does not warrant immediate public health intervention. Given this finding was in a raw product, and while it may have accounted for some sporadic cases, we do not think this can account for the large number of cases identified in this outbreak.

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DECLARATION OF INTEREST

None.

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