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An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – sharing molecular information to solve international outbreaks

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SUMMARY

Salmonellosis is a global problem caused by the international movement of foods and high incidence in exporting countries. In September 2001, in an outbreak investigation Australia isolated *Salmonella* Stanley from imported peanuts, which resulted in a wider investigation in Canada, England & Wales and Scotland. Patients infected with *Salmonella* serotypes known to be isolated from peanuts and reported to surveillance systems were interviewed to determine exposure histories. Tagged image file format (TIFF) images of pulsed-field gel electrophoresis (PFGE) patterns of *Salmonella* isolates were shared electronically amongst laboratories. Laboratories tested packets of 'Brand X' peanuts from various lots and product lines. In total, 97 cases of *S.* Stanley and 12 cases of *S.* Newport infection were found. Seventy-three per cent (71/97) of *S.* Stanley cases were in persons of Asian ethnicity. Twenty-eight per cent of cases recalled eating Brand X peanuts and a further 13% had peanuts in their house in the previous month or had eaten Asian-style peanuts. Laboratories isolated *S.* Stanley, *S.* Newport, *S.* Kottbus, *S.* Lexington and *S.* Unnamed from Brand X peanuts. Isolates of *S.* Stanley from peanuts and human patients were indistinguishable by PFGE. This international outbreak resulted from a product originating from one country affecting several others. Rapid sharing of electronic DNA images was a crucial factor in delineating the outbreak; multinational investigations would benefit from a harmonized approach.

INTRODUCTION

Salmonella enterica is ideally suited to causing widely disseminated outbreaks of gastroenteritis due to the ubiquitous nature of the pathogen, and its ability to survive in products that have a long shelf life [1]. Globally there is massive movement of food products, along with an increasing incidence of salmonellosis in many countries. This has resulted in several international outbreaks caused by a variety of serotypes [2], including: Enteritidis, Typhimurium, Anatum, Virchow, Hadar, Paratyphi B, Saphra, Javiana and

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Agona [3]. Investigations of these outbreaks have implicated a diverse range of foods; from helva, infant formula milk to chocolate and bean sprouts [4–7]. The sharing of electronic images of DNA profiles from infecting *Salmonella* serotypes has made international investigations easier and more rapid [3].

In July 2001, health agencies in Australia and Canada noted an increase in locally acquired *S*. Stanley infections. Australian investigators conducted hypothesis-generating interviews of cases. Two patients, one of whom was visiting Asian friends, recalled eating 'dry-flavoured Asian style' peanuts during the incubation period of their illness. *S*. Stanley was subsequently isolated from 'Brand X' peanuts. Several other serotypes of *Salmonella* (Newport, Lexington, Kottbus, Unnamed) were also isolated from unopened packets of Brand X peanuts imported from Asia. Implicated batches of Brand X peanuts were recalled in Australia and Canada. Information on the outbreak was disseminated via the ProMed international electronic mailing list, and Enter-net – the European international surveillance network for enteric pathogens [8]. Subsequently human cases of *S*. Stanley infection were identified in England & Wales and in Scotland. Health Protection Agency food microbiology laboratories in England & Wales also isolated *S*. Stanley and *S*. Newport from a similar peanut product produced by the same company. We report the findings from four countries for this international outbreak of foodborne salmonellosis and discuss some of the challenges for these types of investigations.

Table 1. *Number of cases and age/sex distribution of people infected with* S. enterica *serotypes Stanley and Newport who met outbreak case definitions, in Australia, Canada, England & Wales and Scotland*

Country	Serotype	No. of cases	Median age (range)	Male : female ratio
Australia	*S*. Stanley	53	9 (0–82)	1 : 0·7
	S. Newport	2	34 (30–37)	0 : 1
Canada	*S*. Stanley	34	20 (1–78)	1 : 1
	S. Newport	10	31 (1–59)	1 : 1
England & Wales	*S*. Stanley	8	24 (3–62)	1 : 1
	S. Newport	0	—	—
Scotland	*S*. Stanley	2	3·5 (1–6)	0 : 2
	S. Newport	0	—	—
Total	*S*. Stanley	97	10 (0–82)	1 : 0·9
	S. Newport	12	33 (1–59)	1 : 1·2

METHODS

Isolate testing

Isolates of *S*. Stanley and *S*. Newport from foods and patients were identified by standard methods [9, 10] and further characterized by pulsed-field gel electrophoresis (PFGE), using methods reported elsewhere [3]. Investigators in the United Kingdom designated the PFGE profile of the outbreak strain of *S*. Stanley as SSTAXB.0002 and that of *S*. Newport as SNWPXB.0030. PulseNet Canada designated the *S*. Stanley strain as SstX2StanXAI.0001 and the *S*. Newport strain as NewpXAI.0036. Tagged image file format (TIFF) images of the outbreak strain of *S*. Stanley and *S*. Newport were shared amongst participating laboratories via email. Isolates were tested for resistance to ampicillin, cefotaxime, chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, sulphathiozole, tetracyclines, trimethoprim, nalidixic acid and ciprofloxacin, using standard methods [11].

Case definitions

The case definition for the four countries reflected the different methods used to detect and investigate cases. In Australia, the case definition was any person reported to health authorities infected with *S*. Stanley or *S*. Newport who had acquired their infection in Australia after May 2001, and the isolate was sensitive to all antibiotics tested. Health Canada included cases with symptoms of vomiting, abdominal cramps, fever or diarrhoea and a stool culture positive for *S*. Stanley with the SSTAXB.0002 PFGE profile. The Public Health Laboratory Service of England & Wales defined an outbreak-associated case as someone who had acquired *S*. Stanley or *S*. Newport with the characteristic SSTAAXB.0002 or SNWPXB.0030 profiles in England & Wales after July 2001. The same definitions were applied in Scotland.

Investigators in each country reviewed national *Salmonella* surveillance data-sets for cases infected with other serotypes isolated from peanut products that were potentially related to this outbreak.

Case follow-up

Health agencies in the four countries interviewed case-patients, each using standard questionnaires. Investigators asked about the patient's illness,

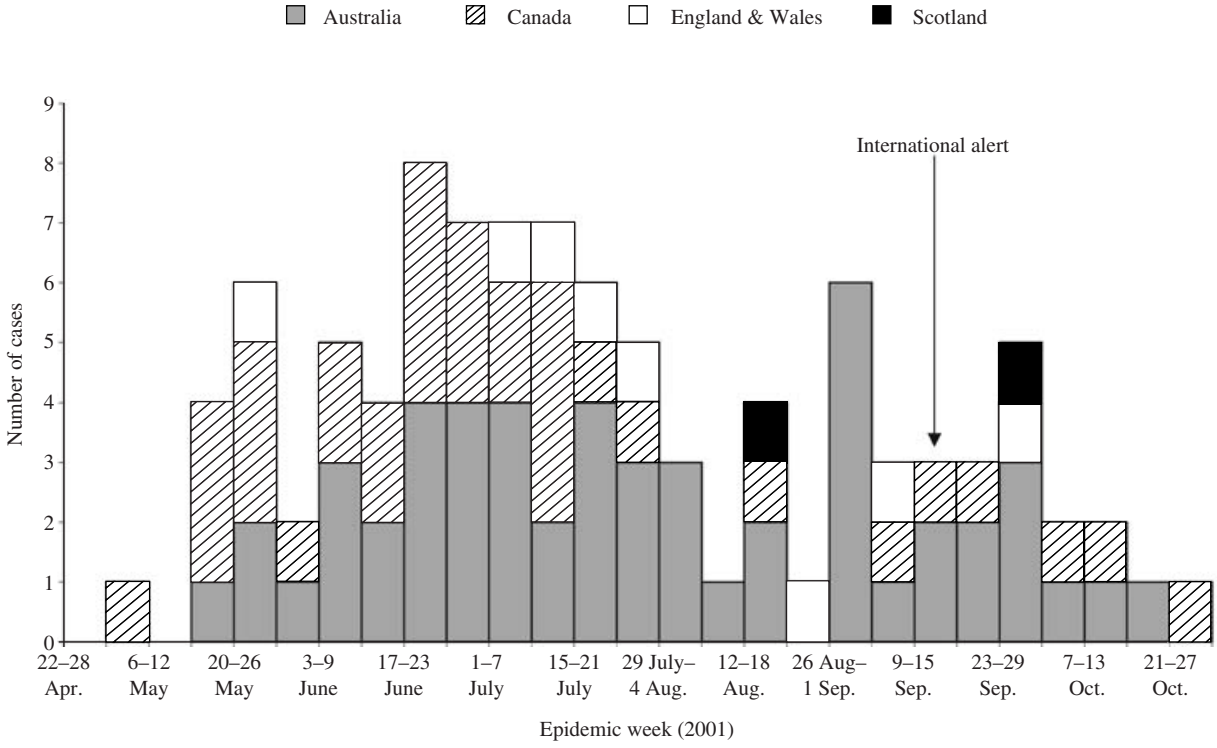

Fig. 1. Outbreak-associated cases of *Salmonella* serotype Stanley in Australia, Canada, England & Wales and Scotland, by epidemic week. Different dates were used to calculate epidemic week for different countries. In Canada it was the date a patient submitted a stool specimen. Australia used the date that a patient's symptoms began. In the United Kingdom the date a faecal specimen was received at the laboratory was used.

demographic details, and exposure to various food items, including imported peanuts. In Australia, case-patients were asked if they had consumed the implicated product in the week prior to their illness or whether they recalled having the specific brand in their house during the previous month.

Food microbiology

In Australia, Canada and England & Wales, laboratories tested samples of different Brand X peanut products, including dry-flavoured peanuts, garlic-flavoured peanuts and roasted peanuts for the presence of salmonellae. Isolation of *Salmonella* from peanuts involved enrichment followed by plating on to selective agars [12–14]. Australia enumerated salmonella in the peanuts using the Most Probable Number (MPN) technique.

RESULTS

Human infections

In total there were 97 outbreak-associated cases of *S.* Stanley infection in the four countries and 12 cases of *S.* Newport infection in Australia and Canada (Table 1). Two cases in Canada were co-infected with both salmonellas. One family in Canada associated with this outbreak had separate members infected with *S.* Stanley, *S.* Newport and *S.* Kottbus. There were no infections with *S.* Lexington, or *S.* Unnamed associated with this outbreak. Outbreak-associated *S.* Newport infections were not reported in either England & Wales or Scotland.

The peak of the epidemic of *S.* Stanley occurred in the last week of August 2001 (Fig. 1). The median age of cases was 11·5 years (0–82 years) and 53% were male. In Australia, 66% (35/53) of patients were of Asian ethnicity, compared to 90% (9/10) in England & Wales and Scotland and 79% (27/34) in Canada. Australia reported four secondary cases in other household members. There was no reported secondary transmission in other countries.

PFGE testing showed that 60 human isolates of *S.* Stanley were the outbreak strain in the four countries (16 Australia, 8 England & Wales, 2 Scotland, 34 Canada). All of these isolates were fully sensitive to all antimicrobials tested and were indistinguishable from isolates obtained from the peanuts

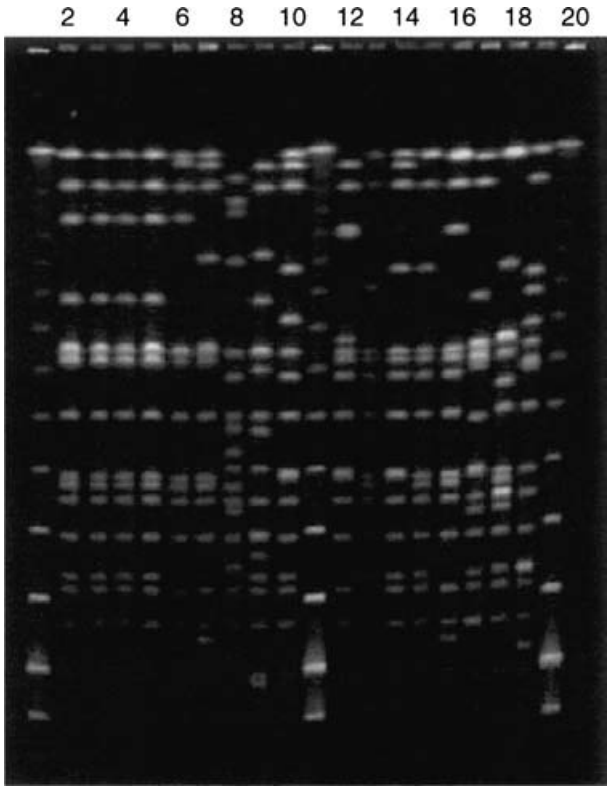

Fig. 2. PFGE profiles of *Xba*I-digested genomic DNA from isolates of *S*. Stanley from peanuts and patients in England & Wales, 2001. Lanes 1, 11, 20, λ 48·6 kb concatamers; lane 2, pulsed-field profile SSTAXB.0002 (patient); lanes 3, 4, STAXB.002 (peanuts); lane 5, SSTAXB.0002 (patient); lanes 6–10, 12–19, SSTAXB3-16 (patients). Voltage, 5·6 V/cm; ramp, 5–60 s; duration, 48 h; agarose concentration, 1·0 w/v. Bio-Rad PFGE grade.

(Fig. 2). In Australia, not all isolates of *S*. Stanley from patients meeting the case definition were characterized by PFGE. England & Wales tested a total of 29 human isolates of *S*. Stanley, which revealed 11 different pulsed-field profile types, while Scotland tested 22 isolates and identified 12 different pulsed-field profile types.

Exposure to peanuts

Overall, 28% (30/109) of 'outbreak-associated' cases ate Brand X peanuts before their illness and a further 13% (14/109) had either had peanuts in their house in the previous month, or eaten unknown brands of Asian peanuts. In Australia, 18 patients reported eating the implicated peanuts within their incubation period, and a further six had the specific brand of peanuts in their house in the month before onset of their illness. In Australia, two patients infected with *S*. Newport recalled eating the implicated peanuts but recall was generally poor. Food histories were difficult to obtain from cases in England & Wales and Scotland due to the time between the recall and interview of cases. Two of the eight cases in England & Wales were known to have consumed these peanuts. A further case reported eating peanuts overseas and another had a packet of unopened product in their house. Neither case in Scotland had a history of consumption of Brand X peanuts. In Canada, 29% (10/34) of the outbreak cases recalled eating Brand X peanuts prior to their illness. One other case had Brand X peanuts in her home, and three additional cases reported eating another or unknown brand of Asian peanut.

Peanuts

Laboratories in the three countries tested a total of 142 unopened packets of Brand X products (42 England & Wales, 75 Australia, 25 Canada) for the presence of salmonellae. All positive products were flavoured or roasted peanuts in their shell. In Australia and Canada, unopened packets of dried flavoured peanuts were positive for *S*. Stanley, *S*. Newport, *S*. Lexington, *S*. Kottbus and *S*. Unnamed (Table 2). In Canada, *S*. Lexington was isolated from roasted peanuts. One opened sample of dried flavoured peanuts retrieved from the home of a Canadian patient infected with *S*. Stanley was found to contain *S*. Stanley and *S*. Newport. Laboratories in England & Wales identified *S*. Stanley and *S*. Newport from packets of garlic-flavoured peanuts of the same batch. The concentration of *Salmonella* was generally very low, ranging from $<0{\cdot}03$ to ~ 2 organisms per gram of peanuts in the shell.

The contaminated products originated from a single Asian country, but were distributed via several major Asian cities. It was impossible to determine the original source of contamination despite inquiries with the country of origin.

Product recall

Brand X products were recalled in each country. In Australia, a product recall was issued for three batches of dry-flavoured peanuts totalling 17 600 packets, although the number recovered from consumers was unknown. One batch of 4200 packets had not been distributed. In the United Kingdom, 22% (3060/13 800) of packets of Brand X of the contaminated batches of garlic-flavoured peanuts were

Table 2. *Brand X peanut products positive for* S. enterica *showing number of batches recalled, total packets, number of items tested and serotypes identified, 2001*

Country	Product	No. of batches recalled	No. of packets in consignments	Total no. of packets recalled	No. of unopened packets tested for salmonellae	No. of packets positive for *Salmonella*	Serotypes identified
Australia	Dry-flavoured dried peanuts	3	17 600	4200	44	12	Stanley, Newport, Kottbus, unnamed
Canada	Dry-flavoured dried peanuts	n.a.	n.a.	n.a.	5	5	Stanley, Newport
	Roasted peanuts	n.a.	n.a.	n.a.*	10	5	Lexington
UK	Garlic-flavoured peanuts	2	13 800	9780	13	6	Stanley, Newport
Total		n.a.	n.a.	n.a.	72	28	

n.a., Not available.
* A total of 8 batches of Brand X products (1 dry flavoured, 4 roasted, 1 garlic flavoured, 2 dried) were recalled in Canada; only two products tested positive as noted above.

recovered from consumers. As a precautionary measure the importer recalled another batch of the same product with a different 'best before' date, and 5340 packets were recovered from the importer's warehouse and 1380 packets recovered from consumers, equating to 93% of the consignment. A total of eight batches of Brand X products were recalled in Canada, including one dry-flavoured product, four roasted, one garlic-flavoured, and two other dried products. The only product lines that tested positive in Canada were two batches of dry-flavoured and roasted peanuts.

DISCUSSION

We identified contaminated peanuts in their shells as the source of an international outbreak of *S.* Stanley affecting Canada, England & Wales, Scotland and Australia, and of *S.* Newport affecting Canada and Australia. The investigation identified a total of 109 cases of *Salmonella* in three continents, arising from a product originating in a fourth continent. Despite finding a range of *Salmonella* serotypes in the products, only human infections with *S.* Stanley, *S.* Newport and *S.* Kottbus were identified. This outbreak illustrates the global potential of foodborne salmonellosis, which is typical of modern day epidemics [15]. It highlights the role and importance of international surveillance systems that can be vital mechanisms in recognizing and investigating such epidemics.

This investigation clearly demonstrates the usefulness of classic *Salmonella* serotyping supplemented by DNA fingerprinting based on PFGE [16]. The international exchange of epidemiological information and TIFF files of pulsed-field profiles was particularly rapid. This circumvented the need to exchange *Salmonella* strains through the postal system, which is expensive and causes long delays in investigations.

One of the limitations of our investigation was that we did not conduct an analytical study to identify the proportion of illness attributable to the peanut products. There were several reasons why investigating teams decided not to conduct a case-control study, including:

- early microbiological confirmation of the vehicle;
- an easily recognized food product with limited distribution;
- poor exposure recall in many cases where infection occurred some months earlier;
- generally mild infections;
- difficulties in identifying an ethnically appropriate control group.

Despite these limitations, it is likely that most patients who were infected with *S.* Stanley and *S.* Newport and who reported consumption of Brand X peanuts acquired infection from eating these nuts. Contaminated products and associated cases in four different countries add further weight to the evidence that peanuts caused this outbreak.

Peanuts and peanut products have been associated with two other outbreaks of salmonellosis, both of which occurred in 1996 [17–20]. The first was an international outbreak of *S*. Agona infections associated with a peanut-flavoured snack originating from Israel, and the second was an Australia-wide outbreak of *S*. Mbandaka due to contaminated peanut butter. It is difficult to test peanuts and peanut products for *Salmonella* when they are imported, as they are ingredients of many different foods. Nuts, such as peanuts and almonds, may be susceptible to *Salmonella* contamination during production, processing and storage [21–23].

There are several challenges for agencies involved in conducting good international investigations of foodborne illness. The first is the need for cooperation and common investigation methods [24]. In this investigation, each country used individual case definitions and assessed exposure slightly differently. Ideally, a lead investigator directing the methodology and protocols to be used would coordinate management of these investigations. Harmonizing investigation methods in foodborne outbreaks can be a challenge within a single country, let alone internationally [25]. Probably the greatest challenge for investigations spanning multiple countries is identifying the original source of contamination. We attempted to determine this in the country of origin, but the contamination was likely to have occurred several months earlier. The details about processing of the nuts in this outbreak were poor, although it appears that they may have been boiled in water with added flavouring before drying.

It was not possible to establish how many contaminated peanuts were prevented from reaching consumers, but it was in excess of 13 980 packets, which would have prevented infections. Different peanut products from the company concerned were contaminated, although at low levels. Very little has been published on the survival of salmonellae in nuts and nut products despite the occurrence of large outbreaks [22, 23].

The control of this outbreak relied on rapid communication of findings and isolate characteristics to international investigators. Sharing of molecular results from foodborne disease investigations is very important and is becoming the mainstay of investigation into outbreaks that are widely distributed geographically [3]. Investigators now have a greater awareness of the international distribution of foods and benefits of sharing molecular typing data on foodborne pathogens. This type of widely distributed outbreak investigation is fairly common amongst collaborative networks, such as Enter-Net [8] and PulseNet [26]. Future investigations will no doubt require more widespread collaboration amongst networks to tackle these international problems. The World Health Organization's proposed 'FoodWeb' initiative could potentially play a role in early warning for foodborne disease of international significance and the coordination of subsequent investigations [27].

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