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A pseudo-outbreak of salmonellosis

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SUMMARY

During July 1991, a single laboratory reported an increased number of an unusual salmonella isolate. An outbreak control team was convened. A case was defined as an individual with diarrhoea from whose faecal sample *Salmonella hadar* was isolated after 1 July 1991. By 30 July, 90 isolates had been identified and 57 persons interviewed including 39 primary cases. Interviews failed to identify any common features among the cases. A review of the laboratory procedures revealed that the selenite enrichment medium was inoculated using the spoon from the stool collection kit after it was used to emulsify the faecal sample with saline for microscopy. *Salmonella hadar* was isolated from this saline. Once this practice was stopped, no further isolates of *S. hadar* were made. This pseudo-outbreak is a powerful reminder to verify the existence of an outbreak, especially when epidemiological data are inconsistent.

INTRODUCTION

An intrinsic part of infectious disease surveillance and any outbreak investigation is to determine whether the reported increase in illness and/or positive laboratory isolates above the expected baseline is real or apparent [1]. Identifying changes in screening procedures, laboratory techniques or in the scientific staff undertaking a particular test is usually straightforward, but changes in the diagnostic and/or reporting behaviour of clinicians, misidentification of organisms and laboratory contamination of specimens can be more difficult to recognize. Relatively few published reports [2–5] of community pseudo outbreaks attributable to laboratory contamination exist, but anecdotal reports suggest they are not infrequent. We report a pseudo outbreak of salmonellosis that occurred in 1991 within the catchment area of one microbiology laboratory.

BACKGROUND

On 18 July 1991, the consultant microbiologist from a laboratory serving a population of approximately 290000 people reported six isolates of apparently

similar strains of a salmonella, provisionally identified as *Salmonella newport* (antigenic structure 6, 8 e, h 1, 2). This was an unusual isolate for the laboratory and the last similar isolate had been in February 1991. The laboratory normally received between 20 and 30 faecal specimens a day and *Salmonella* sp. were isolated from 3 to 6% of these specimens. Neither the total number of specimens received nor the proportion of specimens positive for faecal pathogens had increased in the preceding weeks. The following day a further six similar isolates were reported. Two of these individuals had a dual infection with campylobacter.

The 12 cases were all adults and 8 were female. All the specimens had arrived in the laboratory during the week beginning 15 July. Preliminary enquiries revealed dates of onset of symptoms between mid-June and 15 July and no common exposures. Other microbiology laboratories in the area reported no recent similar salmonella identifications.

A District Outbreak Control Team was convened to determine the cause of the increase in cases and to implement control measures. The isolates were sent to the Laboratory of Enteric Pathogens (LEP), Central Public Health Laboratory, for confirmation of serotyping and phage typing and were identified as *S. hadar*, phage type (PT) 10, an uncommon phage type.

METHODS

Epidemiological

A case was defined as an individual with diarrhoea (three or more loose stools in 24 h) and from whose faecal sample *S. hadar* was isolated after 1 July 1991. Primary household cases were the first in their family to have had a gastrointestinal illness. Secondary household cases were defined as cases who had had household members with a gastrointestinal illness during the 10 days before the onset of their illness.

A questionnaire which included questions on their illness, contact with others who had been ill, travel, attendance at functions and foods eaten, was administered personally to as many as possible of the cases identified between 18 and 26 July. The responses were reviewed. The questionnaire was modified on 26 July to put increased emphasis on the consumption and purchase of the most commonly identified foodstuffs.

Local GPs were informed of the outbreak by two letters, one on 19 July and the second on 25 July. Both letters requested their help in identifying additional cases by the early submission of faecal samples from individuals with diarrhoea.

Microbiological

The prescribed laboratory methods were reviewed to see if any changes had occurred. Records were reviewed to identify and date previous isolations of similar salmonellae both locally and in LEP. All the results for each specimen from which an isolate was made were reviewed.

Sterility checks were made on the selenite enrichment and XLD media. A batch of 17 faecal specimens were recultured.

A detailed review of the actual methods used in processing faecal samples was

made and subsequently the contents of the bottle of saline used to make the faecal emulsion was cultured.

Environmental

Once data from the questionnaire were available, enquiries were made about sources of and distribution networks of commonly mentioned food stuffs, in conjunction with some major retail outlets. Food sampling was undertaken to a limited extent, both in-house by retail outlets and with samples sent to Chelmsford Public Health Laboratory (PHL). The local water undertakers were contacted to ensure that the water supply to the affected areas did not all originate from one source. The Ministry of Agriculture, Fisheries and Food (MAFF) was contacted and asked if *S. hadar* had recently been a problem in poultry, particularly any produced or distributed locally. Details were obtained about the local milk distribution network.

RESULTS

Epidemiological investigations

By 30 July 90 isolates of *S. hadar* had been identified by the local laboratory. They all lived within the laboratory's catchment area. Fifty-seven females and 33 males were affected; 65 of the 76 for whom ages were known were aged over 15 years. Sixty-nine individuals were contacted and 57 of them were interviewed in person using the questionnaires. Thirty-nine primary cases were identified. The other 18 cases were excluded. The exclusions comprised one secondary household case, two cases who had dates of onset prior to April 1991, one case who could not provide a date of onset, four who did not report having had diarrhoea and nine who had spent nights away from home in the week prior to the onset of their illness. Once the nature of the outbreak was recognized, efforts to contact or interview further cases were abandoned.

Detailed information, including food histories was available on 39 primary cases. In two co-primary cases a specimen was not submitted by the other person. One case was admitted to hospital because of the severity of her gastro-intestinal symptoms.

Dates of onset for the primary cases varied between 1 July and 25 July (Fig. 1) with no evidence of a point source pattern. Twenty-six of these cases were female and 32 were aged 15 years or more (Table 1). There was no apparent age clustering. No family outbreaks were reported.

Thirty cases (77%) reported eating poultry, 22 (56%) had eaten cold meats other than ham and 21 (54%) had eaten ham. Twenty cases (51%) had eaten cucumber, 24 (62%) lettuce and 25 (64%) tomatoes. Thirty-six people (92%) consumed milk and 24 (62%) had milk delivered to them at home. Thirty-five (90%) did their main shop at a major retail outlet with no one store predominant and 13 (33%) used a market usually for fruit and/or vegetables. Fourteen (36%) had eaten out (ranging from a full meal to a sandwich) and eight (21%) had eaten barbecued food either prepared at home or at friends. No common food items or supplier, leisure activities or functions were identified from the questionnaire responses.

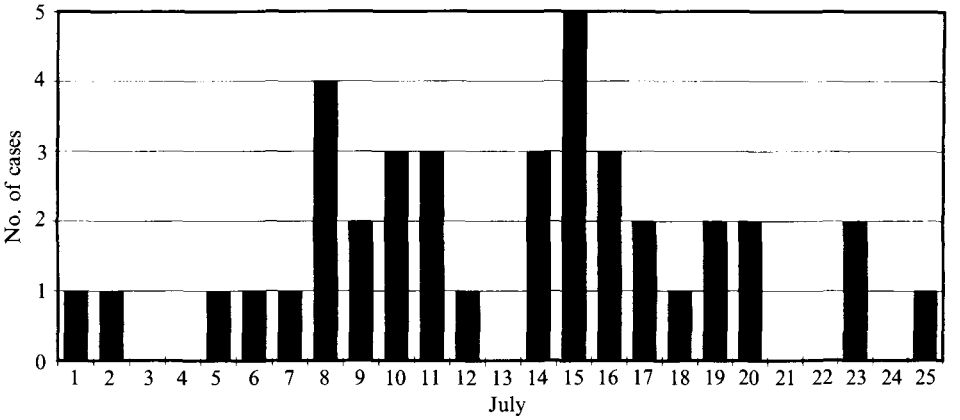

Fig. 1. Dates of onset of illness for 39 primary cases, Essex and Hertfordshire, 1991.

Table 1. *Age–sex distribution of 39 primary cases of* S. hadar

(Essex and Hertfordshire, 1991.)

Age	Male	Female	Total
Under 1 year	1	3	4
1–4	1	0	1
5–14	0	2	2
15–24	2	3	5
25–34	1	7	8
35–44	2	4	6
45–54	2	1	3
55–64	2	1	3
> 65	2	5	7
Total	13	26	39

Microbiological results

Between 18 and 30 July, a total of 90 isolates of presumed *S. hadar* were reported by the local laboratory and 22 had been confirmed by LEP as *S. hadar* PT10. All of those had been positive on subculture of the enrichment medium only and not on the primary XLD plates. No recent changes in prescribed laboratory methods had occurred.

Approximately 80 isolates of *S. hadar* had then been reported by LEP during 1991 of which only two were PT10. One of these originated from another local laboratory in early June but had a different antibiotic resistance pattern. The other was identified during the outbreak, and had a similar antibiotic resistance pattern but the patient had had no contact with the area affected by the outbreak.

An isolate of *S. hadar* PT21 had been made at the local laboratory on 25 June. A further specimen from the patient had been submitted on 12 July and *S. hadar* again isolated. The first isolate was retyped and reported to bear a closer resemblance to PT10 than PT21.

Thirteen cases of the 90 in the outbreak were reported to have dual *Campylobacter* spp. and *S. hadar* infection. During the 2-week period 20 other *Salmonella* spp. were isolated from faecal samples, including 16 *S. enteritidis*.

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The sterility checks on the selenite enrichment and XLD media yielded no growth and the 17 stool samples re-examined yielded results similar to the original findings. On further review of laboratory procedures it was discovered that an unauthorized variation of the prescribed procedure for stool examination had been instituted. The XLD plate was inoculated with a loop and then a sample of stool for microscopy was emulsified on a slide with saline using the spoon from the stool collection kit. This spoon was then used to inoculate the selenite broth after which it was replaced in the stool sample. Normally, both the XLD plate and the selenite broth should have been inoculated before the saline emulsion was made using a loop and not the stool collection kit spoon. On 30 July *S. hadar* and faecal flora but not *Campylobacter* spp. was isolated from the bottle of saline used to make the emulsion of faecal samples.

Of 85 faecal samples sent to the Chelmsford PHL for culture in the 2 days after the contamination was discovered, no isolates of *S. hadar* were made. *Salmonella enteritidis* was isolated from eight samples, *S. brandenburg* from one, *Campylobacter* spp. from four and *Clostridium difficile* from two.

Environmental results

The food samples were negative on routine testing. These results and of those of in-house testing by major retailers was discussed with their Environmental Health Officers and some additional testing was undertaken. Further food sampling was considered but once a potential laboratory contaminant was discovered these enquiries were suspended.

CONTROL MEASURES

As no particular food stuff was identified as a likely vehicle for infection, no specific food warnings or withdrawals were undertaken. However, the importance of good general food hygiene was emphasized to the public, both on an individual basis and in press and other media interviews.

DISCUSSION

The discovery of laboratory contamination as the likely source of most of the positive *S. hadar* isolates led to discussions as to how this had happened, how this information should be handled locally on an immediate basis and on whether the epidemiological results could have led to the contamination being picked up earlier. In addition, the implications for future outbreak investigations were considered.

It remains unknown when and how the saline originally became contaminated. It is possible that more saline was added to a particular slide to obtain a satisfactory preparation and the pipette touched the wet preparation and subsequently contaminated the saline. It is possible, but less likely, that there could have been aerosol spread within the cabinet used while undertaking this work. The saline bottle remained in the cabinet and was only used to make wet preparations. It was replaced irregularly with an autoclaved bottle of saline. The

culture results suggested contamination with faeces rather than a pure culture of *S. hadar*.

It is likely that the first six isolates were all contaminants, the source being the sample received on the 12 July. This source was not suspected until the original isolate had been phage-typed a second time. The unauthorized variation of the procedure for stool examination led to contamination of both the enrichment culture and the stool sample itself which explains why re-examination of 17 stools yielded positive results.

Once the pseudo outbreak had been recognized the local GPs and hospital doctors were informed. Letters were written to all the cases advising to revisit their GP if their symptoms persisted. The GPs and the Health Authority received very few calls from cases. Informal feedback indicated that the patients, the GPs and the public were pleased that the cause had been found and impressed to receive an honest account.

The outbreak generated considerable publicity in the local media. While the cause remained unknown, the emphasis was placed on giving advice about personal and food hygiene.

The prepared final Press Statement explained that it was believed that several different infections were responsible for the reported local increase in gastro-intestinal illness without a single cause being identified. However, the local papers became aware of the laboratory contamination which led to headlines such as 'Lab Foul Up', which were upsetting for staff, although the actual reports were generally accurate.

The possibility of a laboratory cause for the outbreak had been of concern to the microbiologist from the beginning of the investigation particularly as the isolates were only made from the specimens that had been placed in enrichment medium. Initially the report of a similar isolate, from an adjacent laboratory had suggested that the isolates might not be confined to one laboratory but on phage typing this isolate was shown to be dissimilar. A detailed review of laboratory procedures and culture of all fluids and media eventually revealed the source of this pseudo-epidemic; an earlier, detailed review of the procedures whilst they were being carried out would have revealed it sooner but senior staff were spending much time attending the outbreak control committee meetings and were not in the laboratory where much of the technical work was going on.

The epidemic curve did not suggest a point source and interviews with primary cases failed to identify a common exposure. All age groups were affected but no clues were provided by the diets of the youngest cases. Further clues to suggest this was not a true outbreak were provided by the matching of cases with the catchment population of the laboratory and the lack of an increase in the number of specimens submitted to the laboratory until the GPs were requested to consider sampling early on in peoples' illness and there had been local media coverage of the outbreak. Although no foodstuffs were implicated investigation of the food distribution networks did suggest some possible explanations for the geographical distribution of those affected.

Contamination of laboratory equipment and bronchoscopes have been reported in earlier pseudo-outbreaks [6–9]. Other reported pseudo-outbreaks have been associated with atypical mycobacteria in a hospital water supply [10], misidentifi-

cations of *E. histolytica* [11], cryptosporidial oocysts [12], possible technical faults in the technique for taking blood cultures [12–16] and misinterpretation of laboratory tests for infectious mononucleosis and TB [3, 17]. A subsequent review has reiterated the need to ensure that an increase in isolates is real during the preliminary investigation of an apparent outbreak [5].

A sudden increase in positive and similar laboratory isolates requires early investigation in order to try to identify a vehicle of infection and for appropriate public health measures to be instituted. In this respect this pseudo-outbreak was no different to a real outbreak. Those involved are now more aware of the potential for pseudo-outbreaks and the suggestive epidemiological and microbiological features such as the isolation of the organism only in one laboratory especially without a very local suspect source, isolation only from enrichment media, a higher than normal proportion of dual isolations and no simultaneous increase in samples submitted.

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