A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls

Abstract

Between April and June 1996, 15 persons with *Salmonella enterica* serovar Mbandaka infection were reported in South Australia (population 1.6 million) compared with 12 over the previous five years. To identify a possible source for the infections a case control study was conducted.

Methods: Trained interviewers asked 15 cases and 45 controls about their consumption of 105 foods. Controls were matched to case residential location and age. They were selected from a previously constructed database of 3,014 randomly selected South Australian households.

Results: Thirteen of the 15 cases ate 'generic' or 'retail store' brands of peanut butter produced by the same factory in another state, compared with five of the 45 controls ($p<0.01$). *Salmonella* Mbandaka was isolated from three opened jars of peanut butter from case households, and from three unopened jars from retail outlets. Further investigation implicated roasted peanuts from a third Australian state as the source of the *Salmonella* contamination.

Discussion: This is the first recorded outbreak of salmonellosis resulting from the consumption of peanut butter. The SA outbreak investigation comprised a matched case control study to identify possible common food sources. Such investigations need be conducted rapidly to maximise public health benefits, and the utility of selecting controls from a population based database can improve timeliness.

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*S*almonella enterica serovar Mbandaka (*Salmonella* Mbandaka) was first identified as a cause of human infection in Australia in 1978.[1] Between 1985 and 1995, there were 193 cases of *Salmonella* Mbandaka reported to health authorities in Australia.[1] In South Australia until 1994, only three cases of *Salmonella* Mbandaka had been reported, all associated with foreign travel. This situation changed in 1995 when eight of the nine reported cases were locally acquired. Hypothesis generating interviews conducted with these cases were unproductive.

In 1996, the Communicable Disease Control Branch (CDCB) of South Australia identified an outbreak of *Salmonella* Mbandaka infections with 15 cases reported between April 4 and June 14. Authorities in other Australian states had also noted an increase in the numbers of persons reported with *Salmonella* Mbandaka infections.[2] As part of the investigation of this nationwide outbreak we conducted a case control study in South Australia using a methodology new to Australia, with the selection of controls from a previously constructed database. In this report we describe the methodology and findings of this study.

Methods

Persons with *Salmonella* Mbandaka infection were identified from laboratory reports. We defined a case as the first person within a household with a faecal sample yielding *Salmonella* Mbandaka and with gastrointestinal illness between April 1 and May 30, 1996. Infections acquired overseas were excluded.

Controls were selected from a previously constructed database called the Social Environmental Risk Context Information System ('SERCIS')[3] made up of 3,014 South Australian households. SERCIS is a telephone recruiting system that had been used for health surveys in April and May 1996. Households had been selected at random from the electronic version of the telephone directory, which incorporates 81% of South Australian households. One adult, selected at random, residing at these households had participated in one of the previous health surveys in 1996 (response rate 76%) and 95% had consented to participate in future telephone health surveys. Thus, the database was drawn from a sample of 58% of all South Australian households.

Three controls were matched to each case by age and the case's residential location. Controls were selected from households in the same or geographically adjacent postcode area to the case household. Cases aged six months to nine years were matched with controls over the age of six months and within one year of the case's age. Cases aged 10-19 years were matched with controls within two years, and cases greater than 20 years in age were matched with controls within five years of the case's age. Controls were excluded from participation if they had had diarrhoeal illness, defined as three or more loose bowel motions in one day in the months of April or May, overseas travel in April or May, or resi-

dence outside South Australia for more than two weeks during April or May.

Hypothesis-generating interviews with five cases did not implicate a possible common source for the infections, so a comprehensive questionnaire was used in the case control study.

A structured food preference questionnaire was used to interview all cases and controls. Questions focused on foods eaten during a normal week during April or May 1996. Cases and controls were asked which of 105 foods they ate daily, at least once weekly or never during a normal week. Methods of cooking, freezing and defrosting of meats were recorded, as were preferred methods of cooking eggs. Locations of places where food was purchased, such as retail outlets and outlets selling ready-to-eat cooked food were also documented. Brand names of products were recorded where possible. Medications, both prescription and over-the-counter were recorded. Demographic characteristics such as date of birth and address were recorded, as were details of the illness experienced by cases.

Using the same structured questionnaire, staff of the CDCB interviewed cases between June 13 and June 20. Professional health interviewers interviewed all controls on June 23, except for one, who was interviewed on June 26. All interviews were conducted by telephone with the case or control, or with the primary caregiver if the case was under 10 years of age.

Matched quadruplet analyses in Epi Info version 6.02[4] were used to determine associations between foods and illness. Mantel-Haenszel matched odds ratio (OR) with exact 95% confidence limits (CI) was used to determine the level of association. Where the matched odds ratio could not be defined, point estimates and 95% lower confidence intervals were calculated using Stat Logic Exact.[5,6]

Microbiology

Human faecal specimens and food samples were submitted to the Infectious Diseases Laboratory, Institute of Medical and Veterinary Science (IMVS) in Adelaide. Samples were investigated for the presence of *Salmonella* spp. using Australian Standard method AS 1766.2.5-1991. Isolates of presumptive *Salmonella* spp. were biochemically confirmed using Microbact 24E (Medvet Science) and serotyped in the Australian *Salmonella* Reference Laboratory at IMVS using Serobact typing reagents (Medvet Science).

Results

Descriptive epidemiology

By June 13, 15 cases of infection due to *Salmonella* Mbandaka had been identified from faecal isolates. All were primary cases and participated in the case control study. Seven (46%) of the cases were female. Ages ranged from 11 months to 37 years of age, with a median age of four years. Eleven cases were five years of age or less. Cases were geographically widespread throughout South Australia. Onset dates of illness ranged from April 1 to May 22 (see Figure 1). Thirteen cases had diarrhoeal symptoms, five of these with blood in the faeces, 11 cases had abdominal cramps and nine had fever. Other symptoms recorded were nausea (4), vomiting (5), flatulence (5) and anorexia (4). Duration of illness ranged from two
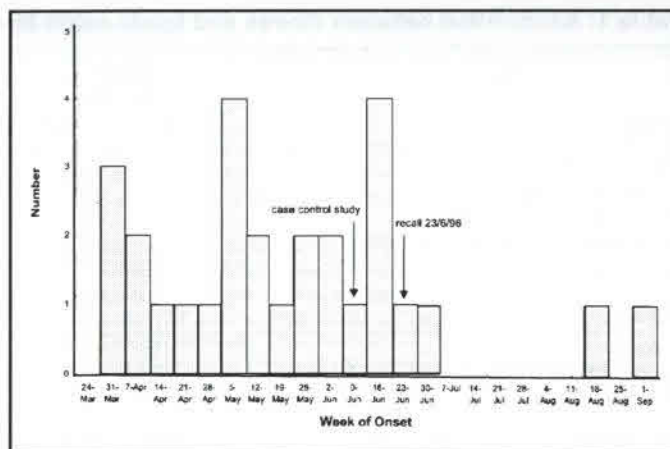

Figure 1: Notifications of *Salmonella* Mbandaka infection, by week of symptom onset in South Australia, 1996.

to 38 days with a mean of 11 days. Three cases were hospitalised.

Interviews were completed with 45 controls (three controls per case). Controls were identified from the 'SERCIS' database with 4-10 possible controls matched for each case. At the conclusion of the interviews, 53 persons had been contacted, with four excluded due to a history of diarrhoeal illness during the study period, and four declined to participate. Seventeen (37%) controls were female. Ages ranged from nine months to 40 years of age, with a median age of four years.

Analytical epidemiology

Foods eaten by cases and controls were analysed by type, brand name and place of purchase. The cases and controls ate nine different brands of peanut butter, all of which were produced outside the state. All but one were produced in the same factory. Four were produced for retail stores and were labelled with the retail store's own brand. We defined these four brands as 'generic brands'. These 'generic brands' were eaten by 13 of the 15 cases compared to five of the 45 controls (p<0.01).

Two other foods – buns and hot chips – showed a statistical association with illness (see Table 1) but, when analysed by brand name, no association was indicated.

Microbiology

As questionnaire information became available during the case interviews, we performed ongoing analyses of the results. We analysed by brand names and place of purchase and considered peanut butter to be a possible source for the infection. Based on these interim results, two opened jars of peanut butter were obtained from case households prior to interviewing controls. Four similar unopened jars were obtained from retail outlets. All were submitted for microbiological testing. Both opened jars and two unopened jars contained *Salmonella* Mbandaka in 25 gram aliquots. One of the unopened jars contained *Salmonella* Mbandaka at a level of four organisms per gram, while the others contained less than three organisms per gram. As a result, further jars were tested. A total of 10 opened jars and 33 unopened jars were submitted for testing. Four of the opened jars were from households where a person had

Table 1: Association between illness and foods eaten by cases and controls.

Food	Case (n=15) Ate	Not ate	Control (n=45) Ate	Not ate	Matched odds ratio (95% CI[a])	
Peanut butter	14	1	31	14	6.5	(0.9 – 119.3)
– Crunchy peanut butter	4	11	11	44	1.4	(0.3 – 5.1)
– Smooth peanut butter	9	6	19	26	2.7	(0.6 – 11.6)
– Generic brand of peanut butter	13	2	5	40	42.4[b]	(95%lower CI = 6.9)
Hot chips	14	1	28	17	15.0	(1.3 – 525.5)
Buns	13	2	19	26	6.0	(1.4 – 64.4)

Notes:
(a) 95% confidence interval.
(b) Calculated using StatXact Logic Exact.[6] Upper 95% CI not estimable.

a positive faecal culture for *Salmonella* Mbandaka and three of these (including the two previously mentioned) contained *Salmonella* Mbandaka. Three unopened jars, representing two different brands and three different batch numbers, contained *Salmonella* Mbandaka.

The factory producing the peanut butter was located in another Australian state and the health officials of that state mounted an investigation into food handling procedures in the factory. Indeed, just prior to the laboratory confirmation of contamination by South Australian laboratories, Victorian laboratories also demonstrated the contamination of peanut butter with *Salmonella* Mbandaka. ELISA tests on unopened samples of peanut butter held by the company supported the evidence of contamination.

Control measures

An Australia-wide recall of nine of the 10 brands of peanut butter produced at the factory was voluntarily instituted on the evening of June 23. The following day, the recall was extended to include all brands of peanut butter produced at the factory. A total of 28 cases of *Salmonella* Mbandaka infection were reported in South Australia for 1996. Only three of these had an onset date after the national peanut butter product recall date.

Discussion

This is the first recorded outbreak of salmonellosis associated with the consumption of peanut butter. The outbreak was detected through surveillance of routine reports of *Salmonella* infections from laboratories and medical practitioners. Normally, case interviews would be used to implicate a source of the organism, and that hypothesis would be tested in a case control study. In this instance, we could not identify a possible source from initial case interviews, and decided to use an extensive food preference questionnaire in the case control study. Interim results of the food preference questionnaire led to the microbiological testing of this food not previously regarded as a likely source for *Salmonella* outbreaks. The case control study also implicated a strong association between *Salmonella* Mbandaka infection and the consumption of 'generic brands' of peanut butter.

Case control studies are useful tools to identify common aetiological factors in outbreak investigations,[7,8] with benefits being realised when timely intervention can prevent further cases.[9] To identify possible causes in a timely fashion, case control studies are often instituted on the basis of small numbers of notified cases, as

in this investigation with 15 cases. Small numbers of cases may present problems analysing associations of foods with illness. As such, to improve the statistical power of this study to detect associations between foods and illness we matched three controls per case. The matching criteria used were the age distribution and geographical residential locations of cases, as these represented unique features of this outbreak, compared with other previous *Salmonella* outbreaks. To account for these variables by stratification in an unmatched design may have reduced the power to detect a significant level of association.

However, the rapid selection of matched controls for studies conducted in the field presents a difficult problem. This study demonstrated the utility of selecting controls from a previously constructed population based database. Controls matched by residential location and age can be selected within several hours. Of the 53 persons contacted, only eight (15%) did not participate in the study. Combined with the use of professional health interviewers, who interviewed controls in one day, the time taken to conduct case control studies in the field could be considerably reduced.

Control selection from a pre-defined database may have introduced selection or recall bias into the case control study. These persons had agreed to participate in future health studies and as such may not be representative of the general population, however the expediency with which controls were selected, and the likelihood of a high participation rate, surpassed other considerations. Other possible sources of controls were the selection of a friend of the case, a neighbour, selection from the electoral role or selection of persons accessed via telephone random digit dialling. From any of these other sources we would have had no knowledge of the representativeness of these controls.

During the investigation, interim analyses of case questionnaire results directed the microbiological testing of peanut butter. The case and control interviews occurred at the same time as the microbiological testing, so did not influence case or control recall. All of the cases and 44 of the controls had been interviewed before the recall of 'generic brands' of peanut butter was publicised. Interviewers were also unaware of the possible contamination of peanut butter at the time of interview.

Public health action may need to be taken pending the discovery of confirmatory microbiological evidence.[10] It was important to continue the case control study and complete interviews with controls, even after *Salmonella* Mbandaka had been demonstrated

in two opened jars of peanut butter in another state. The recall of peanut butter was based on positive ELISA tests of unopened sample jars held at the factory.[2] It was not until the day after the 44 controls and 15 cases had been interviewed that the first positive identification of *Salmonella* Mbandaka using Australian Standard microbiological methods was made in unopened jars of peanut butter.[2]

The peanut butter contamination was not prevented by the factory's quality control program and was only detected by epidemiological investigation of human cases reported with *Salmonella* Mbandaka infection. The level of contamination in both opened and unopened peanut butter jars of less than three organisms per gram was low, but sufficient to cause infection. Low infective doses of *Salmonella* in fatty foods, such as chocolate and cheese, have been previously reported to be associated with human salmonellosis.[11,12] The ultimate source of the *Salmonella* Mbandaka was implicated as roasted peanuts supplied by a peanut roasting factory in a third Australian state. This outbreak highlighted the need for quality control programs such as hazard analysis critical control points (HACCP)[13] throughout the production process.

Even with uniform quality control programs regulating food manufacture, it is unlikely to guarantee the absolute prevention of food contamination. The ability to conduct foodborne outbreak investigations rapidly using only a few cases is paramount to timely intervention and prevention of further cases. To improve timeliness of case control studies, public health units should consider using databases such as SERCIS to allow the rapid selection and interviewing of controls.

Acknowledgments

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