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Stephen Harfield, Rebecca Beazley, Emma Denehy, Alessia Centofanti,  
Paul Dowsett, Tambri Housen and Louise Flood

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Communicable Diseases Intelligence is produced by:  
Health Protection Policy Branch  
Office of Health Protection  
Australian Government  
Department of Health  
GPO Box 9848, (MDP 6)  
CANBERRA ACT 2601

## Email:

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# An outbreak and case-control study of *Salmonella* Havana linked to alfalfa sprouts in South Australia, 2018

Stephen Harfield, Rebecca Beazley, Emma Denehy, Alessia Centofanti, Paul Dowsett, Tambri Housen and Louise Flood

## Abstract

An epidemiological investigation and a retrospective case-control study were conducted into an outbreak of *Salmonella* Havana in alfalfa sprouts, in Adelaide, Australia. In total, 31 cases of *S. Havana* were notified during June and July 2018 and linked to the outbreak. Eighteen cases and 54 unmatched controls were included in a case-control study. Results from the case-control study indicated an increased risk of illness linked to the consumption of alfalfa sprouts; this was supported by trace-back, sampling and environmental investigations. This outbreak of *S. Havana* was caused by consumption of alfalfa sprouts from one local sprouts producer. It is unclear as to when in the production of alfalfa sprouts the contamination occurred. However, contaminated seeds and poor pest control are the most likely causes. This investigation highlights the importance of ensuring that producers take appropriate action to minimise the likelihood of contamination and to comply with legislation and standards for primary production and food safety.

Keywords: *Salmonella* Havana, alfalfa sprouts, outbreak, case-control study, South Australia

## Introduction

Salmonellosis is commonly associated with foodborne outbreaks. Outbreaks are often linked to infected produce, animals and contaminated animal feed used in food production.<sup>1</sup> The incubation period for salmonellosis is 6 to 72 hours, usually 12–36 hours. Symptoms may include fever, diarrhoea, loss of appetite, headache, stomach cramps, nausea and vomiting.<sup>1</sup> Salmonellosis may be particularly severe in young children, the elderly and people with immune suppression.<sup>1</sup>

In Australia, *Salmonella* is a nationally notifiable disease and accounted for 33.4% of all foodborne notifications in South Australia (SA) between 2013 and 2017.<sup>2</sup> During June 2018, a notable increase in *Salmonella* Havana (*S. Havana*) notifications was detected through routine surveillance by the Communicable Disease

Control Branch (CDCB), South Australian Department for Health and Wellbeing (SA Health). Notifications of *S. Havana* are relatively infrequent in Australia. During the period 2013–2017, three to fourteen *S. Havana* cases were reported annually to CDCB. No previous outbreaks have been attributed to *S. Havana* in Australia.<sup>2</sup> Internationally, only one other outbreak reported in 1998 in the United States of America (USA) linked *S. Havana* to alfalfa sprouts.<sup>3</sup> *S. Havana* has also been previously identified in poultry<sup>4–7</sup> and feedstock.<sup>6,8,9</sup>

This investigation included a description of the outbreak, a retrospective case control study, microbiological investigation, and trace-back that led to the identification of cases and the prevention of further cases.

## Method

A descriptive epidemiological investigation and a retrospective case-control study were conducted. This epidemiological investigation was covered by the *South Australian Public Health Act 2011* (the Act) and approval from the Australian National University Human Research Ethics Committee (2017/909). Informed consent was obtained from all participants. A case for the outbreak investigation was defined as laboratory-confirmed infection with *S. Havana* in SA reported to CDCB from 1 June until 31 July 2018.

### Epidemiological investigation

In South Australia, reporting of notifiable conditions to the CDCB, by medical practitioners and diagnostic pathology services, is required under the Act. Notifications are monitored by CDCB staff to determine whether further investigations are required.

Interviewing of laboratory-confirmed salmonellosis cases was conducted by trained CDCB staff using the OzFoodNet *Salmonella* Hypothesis Generating Questionnaire (HGQ).<sup>10</sup> The questionnaire collected information on demographics, onset of illness, symptoms, recent travel, environmental exposures, food history, and locations where food was purchased during the seven days prior to illness onset. Information from the hypothesis-generating interviews informed the case-control hypothesis and was used to assist trace-back.

### Case-control study

A case-control study was conducted to test the hypothesis that illness was associated with consumption of frequently identified foods from the hypothesis-generating questionnaires.

A case for the case-control study was defined as a laboratory-confirmed case of *S. Havana* in SA reported to CDCB from 1 June until 20 June 2018. A case was eligible for the case-control study if initially interviewed with the HGQ

and interviewed with the case-control study questionnaire prior to the media announcement regarding the outbreak on 20 June 2018. Cases not interviewed with the case-control study questionnaire prior to the media announcement were excluded. Controls were recruited using a list randomly generated from the South Australian Monitoring and Surveillance System (SAMSS) survey, a population health survey that monitors trends in health risk factors and chronic disease.<sup>11</sup> The survey is collected monthly from about 600 adults and children using a Computer-Assisted Telephone Interviewing system. Participants must be residents of SA with access to a telephone (including mobile phone). The sampling strategy uses a 'dual overlapping sampling technique applied (mobile phone 70%: landline 30%) through random digit dialling'.<sup>12</sup>

An unmatched control sample was calculated by the Prevention and Population Health Branch of SA Health using the 2018 SAMSS recontacting spreadsheet. Sample size was estimated using the percentage of cases consuming sprouts in the hypothesis-generating study and data from the Victorian Food Frequency study, which estimated the prevalence of eating alfalfa sprouts among healthy community controls. Controls were interviewed by telephone by trained interviewers between 9:30 am and 2:00 pm on 20 June 2018. Controls were excluded if they were not reached prior to the media announcement at 2 pm on 20 June 2018, reported being ill, had returned from interstate or overseas within the last seven days or if another member of the household had an onset of diarrhoea in the two weeks prior to the onset of diarrhoea in the laboratory-confirmed control-case selected for the study. Only one attempt by telephone was made to contact each control.

### Statistical analysis

Data obtained from the case-control study questionnaire were entered into a Microsoft Excel® 2016 database before analysis using Stata® version 15. Univariate analysis was conducted on all food exposures, and generated crude odds ratios, *p*-values and 95% confidence intervals

(exact). The statistical significance threshold was 5%. Categorical variables were assessed via chi-squared test (sex) and continuous variables via *t*-test (age).

### Trace-back, sampling and environmental investigation

Information from hypothesis-generating interviews informed trace-back, sampling and environmental investigations. Business and retailer records were used to identify common suppliers and product producers. Environmental and product samples were collected during environmental inspections. Retail samples of products were collected in the marketplace. All samples were submitted for microbiological analysis.

Investigations conducted by local government council environmental health officers (EHO) as authorised by the *Food Act 2001* (SA),<sup>13</sup> at a hotel implicated in the outbreak, included inspection of kitchen facilities and observation of food preparation procedures. Details of staff illness, absenteeism, and product suppliers' details were requested. Food samples were collected.

Additional inspections of businesses implicated by cases were carried out by the Food and Controlled Drugs Branch, SA Health (FCDB) and by the Department of Primary Industries and Regions, South Australia (PIRSA) through trace-back of distributors and product producers. Environmental and product samples were collected from the production site at the time of this inspection. Several follow-up inspections of the producer were conducted by FCDB and PIRSA.

Food samples and environmental samples were sent to SA Pathology, SA Health, for microbiological analysis using standardised methods.<sup>14</sup>

## Results

### Epidemiological Investigation

In total, 31 cases of *S. Havana* were notified to the CDCB from 1 June to 31 July 2018 and linked to the outbreak investigation (Figure 1),

comprising 19 females (61%) and 12 males (39%), with an age range of 22–87 years and a median age of 65 years. Cases were from both rural SA (17 cases) and metropolitan Adelaide (14 cases). The most frequently reported symptoms were diarrhoea (97%), lethargy (94%), abdominal pain (81%), nausea (77%), fever (74%), headache (68%), muscle ache (61%) and vomiting (42%). The days unwell ranged from 2 to 23 days with a median of 8. Thirteen (42%) cases were hospitalised.

The hypothesis-generating interviews were conducted between 13 and 19 June 2018 with 17 cases. The interviews identified eight cases with the same hotel (Hotel X) as a common exposure. No meals were common between the eight cases. Foods identified from hypothesis-generating interviews as frequently consumed included carrots, apples, bananas, pasteurised milk, cheese, potatoes, tomatoes, chicken and avocado. Five cases who did not eat at the hotel identified eating alfalfa sprouts purchased from one of the following: a supermarket, bakery, fruit and vegetable store, or health food store.

### Environmental investigation

Local government council EHOs inspected Hotel X on 14 June 2018. The EHOs identified alfalfa sprouts were served as a garnish on all hot meals, along with snow pea shoots and mesclun lettuce. Seven food samples were collected, four alfalfa sprouts samples (two from open bags and two from closed bags), one mesclun lettuce leaves sample and two snow pea sprouts samples. Overall, the inspection showed that general compliance with the Australian and New Zealand Food Standards Code<sup>15</sup> was satisfactory. One staff member, a food handler, was identified as being unwell; however, it was unclear if the food handler had worked while ill. No stool sample was obtained for this person. Hotel X used alfalfa sprouts supplied by a local distributor who sourced the alfalfa sprouts from one local sprouts producer (producer A). Trace-back of alfalfa sprouts implicated by cases not linked to Hotel X were also conducted with a supermarket, bakery, fruit and vegetable store,

Figure 1. Epidemiological curve of S. Havana notifications in South Australia by date of onset, 29 May – 23 June 2018

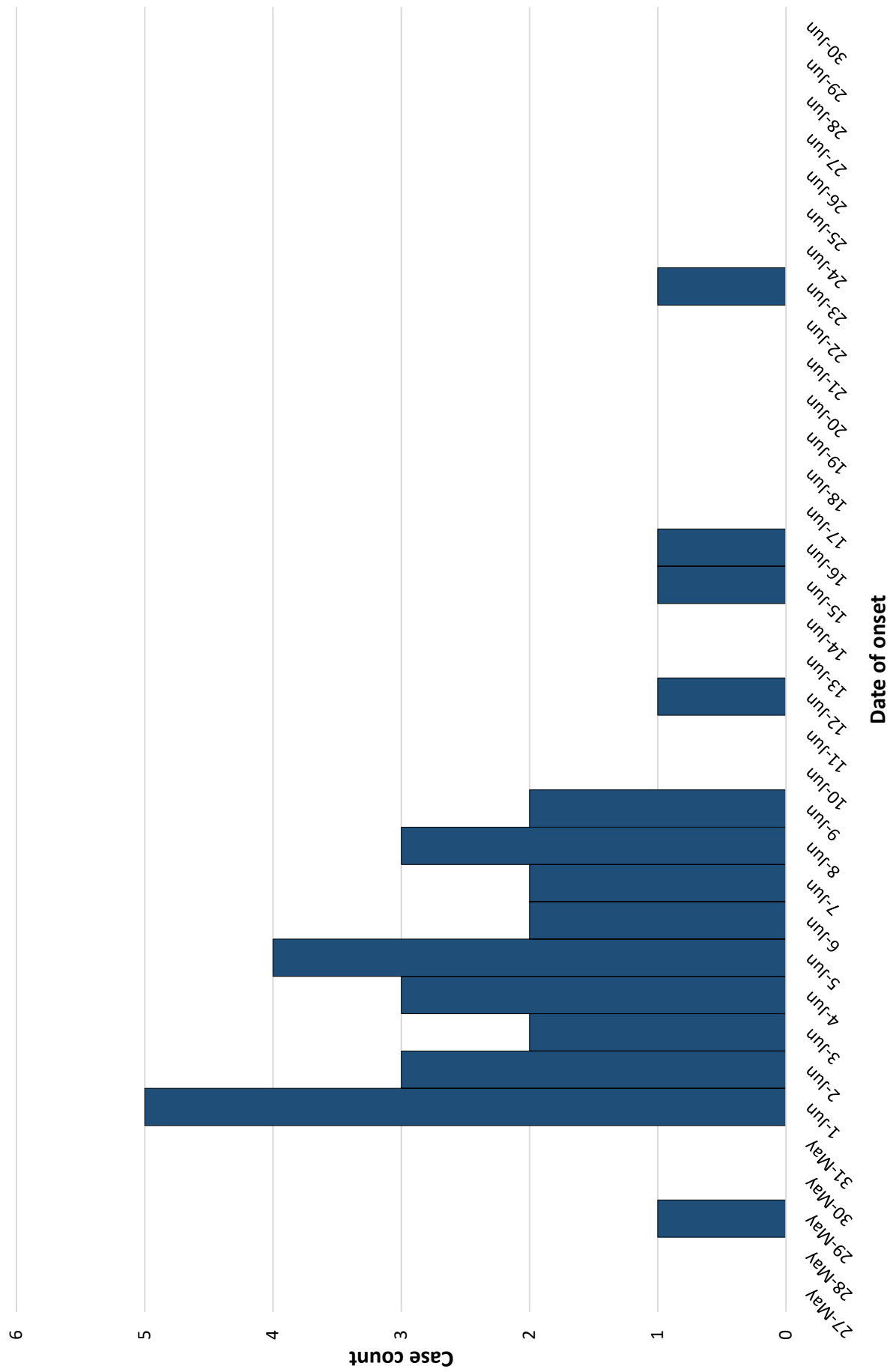


Table 1. S. Havana outbreak – relative risks for food exposures, South Australia, 1–20 June 2018

Exposure	Cases (n=18)		Control (n=54)		Univariate analysis		
	Exposed	%	Exposed	%	Crude OR <sup>a</sup>	95% CI <sup>b</sup>	p-value
<b>Alfalfa sprouts</b>	<b>6</b>	<b>33.33</b>	<b>1</b>	<b>1.85</b>	<b>26.00</b>	<b>2.62–1217.60</b>	<b>&lt;0.001</b>
Black pepper	6	33.33	38	70.37	0.21	0.06–0.75	0.02
Cauliflower	8	44.44	20	37.04	2.67	0.79–9.44	0.07
Capsicum	5	27.78	27	50.00	0.42	0.10–1.51	0.14
Eggs	12	66.67	46	85.19	0.42	0.10–1.95	0.18
Avocado	9	50.00	19	35.19	1.84	0.54–6.21	0.26
Lettuce	11	61.11	27	50.00	1.83	0.52–6.90	0.29
Raw tomato	9	50.00	36	66.67	0.56	0.16–2.00	0.31
Broccoli	10	55.56	36	66.67	0.59	0.17–2.07	0.34
Fresh garlic	4	22.22	16	29.63	0.68	0.14–2.64	0.54
Chicken	7	38.89	27	50.00	0.78	0.21–2.75	0.66
Yoghurt	9	50.00	24	44.44	1.25	0.37–4.18	0.68
Snow pea shoot	1	5.56	2	3.70	1.53	0.02–30.92	0.73
Pumpkin	10	55.56	34	62.96	0.84	0.24–3.05	0.76
Cucumbers	7	38.89	20	37.04	1.08	0.30–3.66	0.89
Raw onions	6	33.33	20	37.04	0.93	0.24–3.26	0.90
Almonds	8	44.44	24	44.44	1.00	0.29–3.32	1.00

a OR, odds ratio

b CI, confidence interval

and health food store. Trace-back identified that alfalfa sprouts were all from the one sprouts producer (producer A).

Random retail sampling occurred on 18 June 2018 and 146 samples of alfalfa and other sprout products were collected. Two South Australian alfalfa producers provided product to the SA marketplace. To ensure a thorough and open investigation both producers were investigated (producer A, producer B). The Food and Controlled Drugs Branch and PIRSA undertook joint environmental investigations at both South Australian alfalfa producers on 19 June 2018. Significant food safety issues at one producer (producer A) included vermin control: an inspection identified vermin faeces underneath pallets in the bulk storage area storing seeds. Fifty-one samples were collected along the production line from producer A: 42 were food and seed samples including a variety of alfalfa and snow pea sprouts, and nine were environment samples including vermin faeces. At producer B, 117 samples were collected from a mix of different sprout products.

Of the seven food samples collected by local council EHOs at Hotel X on 14 June 2018, three alfalfa sprouts samples returned positive results for *S. Havana* on 20 June 2018. Of the random retail samples collected, five returned positive results for *S. Havana* and were from the same sprout producer (producer A). Eleven positive results were returned on sprout product samples collected from producer A, with six *S. Havana* and five *S. Oranienburg*. While *S. Oranienburg* was identified from samples collected from the sprouts producer, no cases of *S. Oranienburg* had been notified in SA since April 2018. No positive results were identified from producer B.

### Case-control study

Eighteen cases were identified and included in the case-control study. Cases included seven males (39%) and 11 females (61%), with an age range 22–87 years and a median age of 69 years. Of the 268 potential controls contacted, 54 unmatched controls were eligible to be enrolled

into the study. Exclusion of controls was due to individuals being non-contactable (96%), reportedly ill (3%) or reportedly having travelled recently (1%). Controls included 19 males (30%) and 35 females (70%), with an age range 21–94 years and a median age of 71 years. There was no statistically significant difference between cases and controls in age (test  $t = 1.70$ ,  $p$ -value = 0.10), nor in sex ( $\chi^2(1) = 0.53$ ,  $p$ -value = 0.47).

Table 1 shows the results for the univariate epidemiological analysis. Increased risk of illness was shown for alfalfa sprouts (odds ratio 26.0, 95% CI 2.62–1217.60,  $p$ -value <0.001). Multivariate analysis was not conducted as only one food exposure, alfalfa sprouts, was statistically significant ( $p$ -value <0.05) and had a crude odds ratio greater than 2.0 with the 95% confidence interval not crossing unity.

### Public Health Action

Based on epidemiology, laboratory results and trace-back, an emergency order under the *Food Act 2001* (SA) was served on producer A to cease distributing products, which were only distributed within SA, and a consumer level recall of all alfalfa products from the supply chain was issued on 21 June 2018. A media release and public health alert to warn South Australians not to eat alfalfa sprout products from producer A was issued on 20 June 2018.

PIRSA returned to producer A to evaluate the effectiveness of the food recall and conduct a more intensive overview of the facility and processes on 22 June 2018. No breakdown in the production process was identified; however, there were several structural deficiencies identified. Producer A was advised to rectify issues around vermin entry points and remove any equipment that could not be easily cleaned and sanitised, and to review their food safety programme to ensure it adequately addressed critical control points as required under the Food Standards Code (FSC) – Production and Processing Standard for Seed Sprouts (4.2.6). The producer committed to cease the production of all sprout products and to rectify issues identified.

Food and Controlled Drugs Branch returned to producer A on 29 June 2018 to witness the secure destruction of the recalled product.

## Discussion

Consumption of alfalfa sprouts linked to one sprouts producer showed a significant association with illness, an odds ratio of 26.0 (95% CI 2.62–1217.60,  $p$ -value <0.001). The findings and observations from the environmental investigation and laboratory results, which included positive results for *S. Havana* from three samples collected from Hotel X, five samples from random retail samples and six from samples collected from sprout producer A, provided further support to the epidemiological evidence. This was a significant achievement given that the case-control study was conducted in one day. This outbreak was the largest identified *S. Havana* outbreak in Australia to date. In total, 31 cases of *S. Havana* were reported and linked to the outbreak.

Alfalfa sprouts are considered a high-risk product due to the risk of microbial contamination inherent in sprout seeds and production:<sup>16</sup> sprouts require warm and humid conditions to grow, which is also ideal for bacterial pathogens.<sup>17</sup> It is unclear as to when in the production of alfalfa sprouts the contamination occurred. However, contaminated seeds are the most likely cause given their high risk. Alfalfa sprouts are usually consumed raw which increases the risk of human infection. Alfalfa sprouts<sup>18–24</sup> and other sprouts<sup>23, 25–27</sup> have been linked to other *Salmonella* outbreaks internationally. Only one other outbreak was identified in the literature, in 1998 in the USA, which linked *S. Havana* to alfalfa sprouts.<sup>3</sup> This particular outbreak involved 18 cases from California and Arizona and identified contaminated seed as the likely source.

With 13 (44%) cases hospitalised, the hospitalisation rate in this outbreak was high, in comparison to the normal hospitalisation rate for salmonellosis of 21% in 2018 for SA.<sup>28</sup> This suggests that either there might have been a high

dose of contamination on the alfalfa sprouts or the outbreak strain might be more pathogenic than other *Salmonella* strains, thus leading to a higher burden of disease for this specific strain.

To minimise future outbreaks linked to alfalfa sprouts and other sprouts, it is suggested that sprouts producers take appropriate action to minimise the likelihood of contaminated product, including using a decontamination step to minimise the bacterial load on sprout seeds and in the sprouting process and ensuring they comply with legislation and standards for primary production and food safety. In South Australia, all sprouts producers are required to hold accreditation under the *Primary Produce (Food Safety Schemes) Act 2004* and the Primary Produce (Food Safety Schemes) (Plant Products) Regulations 2010; comply with Food Standards Code – Production and Processing Standard for Seed Sprouts (4.2.6); and have an approved food safety arrangement.

There are several limitations to our outbreak investigation. Firstly, recall by individuals of foods eaten is always a concern in a retrospective case-control study, as asking someone to recall what they ate before becoming ill can be a challenge particularly if it has been several days in between. Also, the HGQ asks about what food did you eat, requesting a person to recall the food they consumed, whereas the case-control study questionnaire is framed as ‘did you eat...’, which is more likely to prompt a person’s memory. This might help explain why those who ate at Hotel X did not recall being served alfalfa sprouts on their meal as a garnish when asked about it during the HGQ. However, one case who ate at Hotel X recalled eating alfalfa sprouts when asked using the case-control study questionnaire. Secondly, controls were not matched, however statistically there was no difference between the cases and controls for age or sex. Thirdly, univariate analysis identified alfalfa sprouts were the most likely cause with statistically significant odds ratio,  $p$ -value and confidence interval. However, the confidence interval is wide (2.62–1217.60), which is related to the small sample size.

## Conclusion

This outbreak of *S. Havana* in SA was caused by consumption of alfalfa sprouts from one local sprouts producer. Alfalfa sprouts are considered a high-risk product due to risk of microbial contamination in sprout seeds, and production occurring in an environment which is ideal for growth of bacterial pathogens. In this outbreak it is unclear as to when in the production of alfalfa sprouts the contamination occurred. However, contaminated seeds and poor pest control are the most likely causes. This investigation highlights the importance of ensuring that producers comply with legislation and standards for primary production and food safety and that equipment is adequately maintained to minimise the likelihood of contamination.

## Author details

Stephen Harfield<sup>1,2</sup>

Rebecca Beazley<sup>3</sup>

Emma Denehy<sup>3</sup>

Alessia Centofanti<sup>3</sup>

Paul Dowsett<sup>4</sup>

Tambri Housen<sup>1</sup>

Louise Flood<sup>3</sup>

1. Australian National University, Canberra, Australian Capital Territory, Australia
2. South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia
3. South Australian Department of Health and Wellbeing, Adelaide, South Australia, Australia
4. Department of Primary Industries and Regions, South Australia, Adelaide, South Australia, Australia

## Corresponding author

Stephen Harfield

South Australian Health and Medical Research Institute

PO Box 11060

Adelaide, South Australia Australia 5001.

Phone: +61 8 8128 4000

Email: [stephen.harfield@sahmri.com](mailto:stephen.harfield@sahmri.com)

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