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Original article

A protracted outbreak of *Salmonella* Hessarek infection associated with one brand of eggs—South Australia, March 2017 – July 2018

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Abstract

Salmonella Hessarek is an uncommon serotype in Australia. We report on the investigation of a protracted outbreak of 25 cases of *S.* Hessarek gastroenteritis in which cases were defined as any laboratory confirmed case of *Salmonella* Hessarek notified to the South Australian Communicable Disease Control Branch from 1st March 2017 to 3 July 2018. We conducted a descriptive case series investigation interviewing all cases and 17 (68%) reported consuming brand X free-range eggs. Four samples of one-dozen brand X eggs were cultured for the presence of *Salmonella* spp. One out of the four samples returned positive for *S.* Hessarek in the contents of the eggs; *Salmonella* was not present in any of the whole egg rinses of the four samples. The high proportion of cases reporting the consumption of brand X free-range eggs and the isolation of *S.* Hessarek from sampling four dozen brand X eggs is an unusually strong signal implicating brand X eggs as the source of this outbreak. From a public health perspective, it is important to understand the behaviour of *S.* Hessarek including its ability to be present in the content of eggs and further research is recommended. The findings in this investigation into a rare *Salmonella* serotype highlight the need for continuous monitoring of the epidemiology of *Salmonella* in Australia including the epidemiology of egg-associated *Salmonella* outbreaks of human disease.

Key words: *Salmonella*, Hessarek, gastroenteritis, foodborne disease, eggs

Introduction

Salmonella enterica subspecies *enterica* serotype Hessarek (*S.* Hessarek) is an uncommon serotype in Australia. From 1 January 2012 to 31 December 2016, there were 96 notifications of *S.* Hessarek nationally (the National Notifiable Diseases Surveillance System *Salmonella* public dataset does not include *Salmonella* notified in the Australian Capital Territory), representing 0.1% of Australian *Salmonella* notifications for the five-year period.[1] Of the 96 notifications, 52 (54%) were for residents of South Australia, a state in which 7% of the Australian population resides. For the five year period, the rate of *S.* Hessarek notifications in South Australians was more than seven times higher than the rate for Australians overall; 3.1 compared to 0.4 notifications per 100,000 persons.[2] Globally, there is one published report of a *S.* Hessarek outbreak in humans; in 2005, five cases notified within the Australian Capital Territory were sourced to free range eggs served at a restaurant.[3]

S. Hessarek was originally isolated from a Common Raven (Corvus corax) in Iran in 1953[4] and has subsequently been detected in outbreaks of septicaemic salmonellosis in wild birds (song thrushes and European starlings)[5,6,7] and, in European mammals (lynx,[8] red foxes[9] and free-range pigs[10]), possibly transmitted through ingestion of infected birds.[5]

The South Australian (SA) Communicable Disease Control Branch (CDCB) observed an increase in *S.* Hessarek notifications beginning around mid-2014. Between November 2016 and February 2017, two to six *S.* Hessarek cases per month (Figure 1) amongst a total of 136 *Salmonella* notifications per month had been noted. On 21 March 2017, the CDCB became aware of five cases of *S.* Hessarek notified in the three weeks since 1 March 2017. This was more than the expected 0.8 *S.* Hessarek notifications in March, based on data for the years 2012 to 2016. An investigation commenced to identify any common cause of illness for which appropriate public health action could be implemented to prevent further cases.

Methods

Epidemiological investigation

A retrospective review of previous *S.* Hessarek cluster investigations in SA was undertaken. We extracted outbreak records from 1 January 2001 to 21 March 2017 from the SA OzFoodNet Outbreak Register and the SA Notifiable Infectious Disease Surveillance System, identified investigations of *S.* Hessarek and, reviewed the investigation summaries to look for potential sources of the pathogen.

A case-series investigation was conducted to generate hypotheses about the source of *S.* Hessarek infection in cases notified in SA since 1 March 2017. We interviewed all persons notified with *S.* Hessarek infection between 1 March 2017 and 3 July 2018 using the national OzFoodNet *Salmonella* Hypothesis Generating Questionnaire. The data collected via the questionnaire include demographic details, clinical information including date of illness onset and symptoms experienced, information regarding any contact with persons with gastroenteritis in the seven days prior to onset of illness, travel history, environmental exposures such as animal contact and consumption of untreated water, food items eaten outside of the home, a seven day open-ended food history and specific questions regarding poultry and egg consumption.

Questionnaire responses relating to egg consumption, including brands of eggs consumed, were compared with data from 20 other SA community *Salmonella* clusters which were investigated using the same *Salmonella* Hypothesis Generating Questionnaire between 1 March 2017 and 3 July 2018. This comparison was intended to identify whether the pattern of consumption of particular egg brands reported during the *S.* Hessarek investigation was similar to the pattern of consumption of particular egg brands reported in SA in general. A case-control study was not conducted as we initially anticipated that there would be insufficient cases to generate a meaningful result and, later in the investigation, there was deemed to be sufficient epidemiological and laboratory evidence to identify the source of the outbreak without case-control study evidence.

An outbreak case was defined as any laboratory confirmed case of *Salmonella* Hessarek notified to the South Australian Communicable Disease Control Branch from 1st March 2017 to 3 July 2018. All *S.* Hessarek notifications were confirmed by the Australian Salmonella Reference Centre.

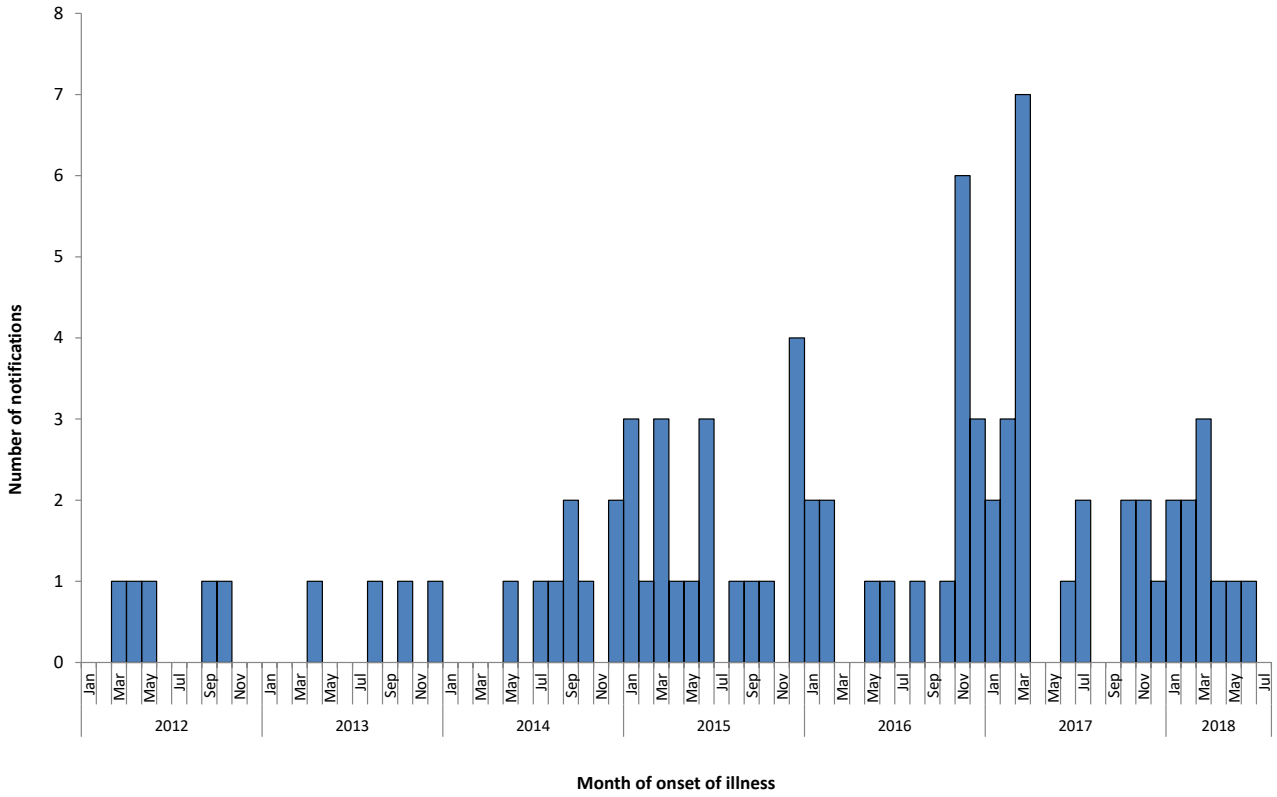
Data were analysed using Microsoft Excel 2010 (Microsoft United States of America (USA)).

The investigation was conducted under the auspices of the *South Australian Public Health Act 2011* and covered under the Australian National University Human Research Ethics Committee approval (2017/909). The SA Department for Health and Ageing Human Research Ethics Committee granted approval for publication of the findings (HREC/17/SAH/113).

Environmental investigation

Based on the responses of seven cases interviewed between 22 March and 7 April 2017, on 10 April 2017 the SA Health Food and Controlled Drugs Branch (FCDB) conducted retail sampling of brand X free-range eggs; four samples of one-dozen eggs with best before dates between 21 April and 12 May 2017 were collected from

Figure 1: Notifications of *Salmonella* Hessarek infection by month of illness onset, South Australia, 1 Jan 2012 to 3 July 2018



two retail stores and cultured for the presence of *Salmonella* spp. by the SA Pathology Food and Environmental Laboratory.

Results

Epidemiological investigation

CDCB had investigated previous clusters of *S.* Hessarek infection in SA; in 2006 a cluster of nine cases of *S.* Hessarek was associated with raw or semi-cooked eggs, in 2014, an investigation of eight cases found that seven cases consumed chicken and six consumed eggs with three cases consuming brand X free-range eggs and, in January 2016, an investigation of six cases found that four consumed eggs, with one case reporting consumption of brand X eggs and another case reporting purchase of eggs of an unknown brand in the isolated SA region where brand X eggs are produced.

Between 1 March 2017 and 3 July 2018, 25 cases met the case definition (Figure 1). The median age of cases was 49 years (age range 1-91 years) with nine cases (36%) aged 71 years or older. Fifteen cases were male and ten were female. Twenty-one cases lived in metropolitan Adelaide and four were non-metropolitan residents. Ten cases (40%) were hospitalised and two other cases occurred in pregnant women.

Twenty-four of the 25 cases (96%) reported eating eggs; 23 cases (92%) consumed eggs at home including five cases (20%) who consumed eggs both at home and away from home and one case (4%) consumed eggs away from home only. Seventeen cases (68%) are known to have consumed brand X free-range eggs, another brand of eggs was named by three cases (12%). Twenty-four cases responded to questions about food items other than eggs and one case declined these questions. Ten cases (42% of 24 respondents) consumed poultry cooked at home and eight cases (33% of 24 respondents) consumed chicken which was purchased cooked; however, there were no chicken items with common brands or from common retail outlets.

Eleven other food items were reported by more than 25% of cases but, with the exception of bottled water, no common brands or common retail outlets were identified. Three brands of bottled water were each named by two to four cases (8-17%). Contact with dogs, cats and/or dried pet food was reported by seven to ten cases (29-42%) but further detail did not indicate any of these exposures as a possible common source (Table 1).

The eggs consumed were cooked in a variety of ways including fried, boiled, scrambled, poached, as omelette and in Béchamel sauce. Seven cases consumed raw egg: in smoothies (three cases), in raw cake batter (three cases) and one case sucked raw eggs (Table 1).

Environmental investigation

One out of four samples returned positive for *S.* Hessarek in the contents of the eggs. *Salmonella* was not present in any of the whole egg rinses of the four egg samples. Additionally, FCDB identified that *S.* Hessarek had been isolated from the content of brand X brand eggs but not the whole egg rinse during a retail food survey in 2014.[11]

Discussion

The high proportion of cases reporting the consumption of brand X free-range eggs and the isolation of *S.* Hessarek from sampling four dozen brand X eggs is an unusually strong signal implicating brand X eggs as the source of this protracted *S.* Hessarek outbreak in South Australia. There is also absence of another possible source based on the hypothesis generating interviews.

Brand X eggs are produced in SA and are predominantly sold in SA. A limitation of the epidemiological investigation is that we did not have information on the proportion of the SA population who usually consume brand X eggs. For reasons stated previously, a case-control study was not conducted and, SA lacks a food frequency consumption database to compare cluster investigation results with the foods that are consumed by healthy people in the community. Egg consumption data, obtained from case interviews in 20 other SA *Salmonella* clusters during the same time period, 1 March 2017 to 3 July 2018, found that only 5/189 (3%) of interviewed persons and 5/125 (4%) of respondents reporting egg consumption, consumed brand X eggs. This suggests that the proportion of cases reporting consumption of brand X eggs in the *S.* Hessarek investigation is not simply a reflection of market share for brand X eggs within SA, however, it is possible egg consumption patterns for persons who have not experienced *Salmonella* infection may differ from those who have and who were interviewed.

The ongoing nature of this outbreak reflects the difficulty in controlling *Salmonella* infection in free range laying flocks. Brand X eggs are produced on a free-range farm and birds raised in free range production systems are potentially exposed to different environmental stressors than caged birds, including social stress and aggression, predation, or thermal challenges with stress known to be a determinant of shedding of *Salmonella*.[12] Additionally, the control of rodents and other potentially infected animals and environments is challenging on free-range farms. *Salmonella* contamination of eggs is a complex issue affected by variables at each stage of the food production process. Currently, the literature regarding the benefits of free range, barn and caged production processes with respect to *Salmonella* contamination is conflicting. However, the current literature does indicate it is not yet achievable to produce eggs guaranteed to be *Salmonella* free.[13]

Because *Salmonella* can be highly persistent in both infected birds and diverse environmental reservoirs, global egg safety programs include interventions at multiple stages of egg production and supply to the public.[14] However, the specific interventions, such as requirements for vaccination of laying flocks against specific *Salmonella* serotypes, requirements for monitoring for *Salmonella* on egg farms, requirements for egg pasteurisation or other actions when *Salmonella* is detected in a laying flock,

Table 1: Food items consumed by cases of *S.* Hessarek infection, South Australia, 1 March 2017 to 3 July 2018

Food Item	Number of cases reporting consumption / Number of cases interviewed *	Proportion of interviewed cases reporting consumption
Eggs	24/25	96%
Eggs eaten at home	23/25	92%
Eggs eaten away from home	6/25	24%
Foods containing raw egg	7/25	28%
Brand X eggs	17/25	68%
Poultry - cooked at home	10/24	42%
Chicken pieces	8/24	33%
Chicken whole	2/24	8%
Chicken on a skewer	1/24	4%
Chicken mince	1/24	4%
Chicken sausages	1/24	4%
Chicken-other	1/24	4%
Turkey	1/24	4%
Duck	0/24	0%
Poultry - purchased cooked	8/24	33%
Roast/barbecue chicken	2/24	8%
Chicken burger	1/24	4%
Shredded chicken	0/24	0%
Other cooked chicken	6/24	25%
Food Items reported by more than 25% of cases		
Bread	18/24	75%
Potatoes	11/24	46%
Bottled water	11/24	46%
Beef (non-minced)	10/24	42%
Coffee	9/24	38%
Bananas	9/24	38%
Pasteurised milk	9/24	38%
Carrots	8/24	33%
Fish	8/24	33%
Lettuce	7/24	29%
Cucumbers	7/24	29%
Environmental Exposures		
Dogs	10/24	42%
Cats	7/24	29%
Dried pet food	10/24	42%

* The number of cases interviewed varies as one case declined interview for all food items except eggs.

requirements for washing of eggs, and requirements for mandatory refrigeration of eggs vary vastly between countries and vary with respect to which specific *Salmonella* serotypes the interventions apply to (often, interventions are applicable to *Salmonella* serotypes *S.* Enteritidis and *S.* Typhimurium only).[14] To illustrate, egg washing with sanitizers is one of the most common methods of reducing eggshell contamination in Australia, Japan, and the USA but the technique is banned in the European Union[15]; the major advantage of egg washing is the removal of faecal debris thereby reducing the overall bacterial load on the eggshell surface. However, the process requires strict control, especially of rinse water temperature and quality, to avoid adverse results including cracking of the shell, damage to the egg cuticle layer and egg penetration by *Salmonella* bacteria.[16]

The variation in egg safety programs internationally results from variation in the perceived risk of contaminated eggs between countries; each country's risk management/tolerance/perception being influenced by local industry, environmental, cultural and epidemiological factors, for example, the types of *Salmonella* contaminating eggs. Stringent egg safety programs in the USA and Europe have resulted from the prevalence of *Salmonella* Enteritidis in those countries. *S.* Enteritidis has the ability to colonize the ovary/oviduct of laying hens for long periods and therefore to internally contaminate eggs, and has been the most frequent serotype associated with egg-related foodborne outbreaks in Europe since the mid-1980s.[17] *S.* Enteritidis is, however, not endemic to Australian commercial layer flocks, a factor which was taken into account during the Food Standards Australia New Zealand (FSANZ) risk assessment of egg production and processing in Australia, a component of the development of the Primary Production and Processing (PPP) Standard for Eggs and Egg Products (Standard 4.2.5), gazetted in May 2011.[18]

In Australia, egg producers must comply with Standard 4.2.5 and under clause 11(1) an egg producer must not sell or supply eggs or egg pulp for human consumption if it knows, ought to reasonably know or to reasonably suspect, that the eggs are 'unacceptable'. 'Unacceptable egg' is defined as: a cracked egg or; a dirty egg; 'egg product' (defined as the contents of an egg in any form) which has not been pasteurised or subjected to heating or other processes that provide a lethal effect on any pathogenic micro-organisms in the egg product; or egg product which contains a pathogenic micro-organism whether or not the egg product has been processed as previously described.[19] The definition of 'unacceptable egg' and clauses restricting the sale of such product do not automatically restrict the sale of whole eggs which are not known to be contaminated but are from a producer or farm where *Salmonella* has been isolated within whole eggs or when a farm or producer has been associated with an outbreak of human disease.

An important consideration in the risk assessment on which the Primary Production and Processing (PPP) Standard for Eggs and Egg Products (Standard 4.2.5) of the Australian Food Standards Code is based is the premise that, in Australia, *Salmonella* contamination in eggs occurs via dirty or cracked eggs. Our finding of *S.* Hessarek in the content of eggs but not in the egg-shell rinse on two occasions, and our association of *S.* Hessarek contaminated eggs with cases of human salmonellosis raise questions regarding whether the risk of contamination of eggs with *Salmonella* in Australia has changed since Standard 4.2.5 was introduced in 2011.

There are two pathways for eggs to become internally contaminated with *Salmonella*: direct contamination occurs during the formation of an egg in the reproductive track of hens (including ovary and oviduct), whereas indirect contamination occurs after an egg has been laid and *Salmonella* contaminating the outside of the egg penetrates through the shell membrane.[20] From a public health perspective, it is important to understand the behaviour of *S.* Hessarek including its ability to be present in the content of eggs and further research is recommended. A high proportion of cases in this investigation were elderly or pregnant and/or consumed raw

egg which suggests that *S.* Hessarek might be an opportunistic rather than highly virulent cause of *Salmonella* infection in humans.

To determine whether cases of *S.* Hessarek notified prior to March 2017 were associated with the source identified in this investigation, we explored the possibility of conducting whole genome sequencing (WGS) of human and egg *S.* Hessarek isolates in SA since 2014. However, as *S.* Hessarek is a rare *Salmonella*, no *S.* Hessarek reference genome, required for the WGS analysis, is available globally. Further research incorporating WGS of *S.* Hessarek would require generation of a complete reference genome for *S.* Hessarek based on consideration of the public health benefit of WGS in understanding the epidemiology of this rare *Salmonella* serotype.

The findings in this investigation highlight the need for continuous monitoring of the epidemiology of *Salmonella* in Australia including the epidemiology of egg-associated *Salmonella* outbreaks of human disease. Any substantial changes in the epidemiology of egg-associated *Salmonella* need to be considered in future risk assessments related to *Salmonella* in eggs and future reviews of the Primary Production and Processing Standard for Eggs and Egg Products (Standard 4.2.5) of the Australian Food Standards Code. Joint efforts from Australian national and state/territory communicable disease control, food safety and primary industry organisations are essential to the control of *Salmonella* in the Australian egg supply chain.

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