RISK FACTORS FOR SPORADIC *SALMONELLA* BIRKENHEAD INFECTION IN QUEENSLAND AND NORTHERN NEW SOUTH WALES: A CASE CONTROL STUDY

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Salmonella Birkenhead is one of the most commonly notified causes of gastroenteritis in southern Queensland and northern New South Wales. It is notified infrequently in other areas of Australia. This limited geographical distribution suggests a possible environmental source. A case control study was conducted to explore risk factors for sporadic infection with *Salmonella* Birkenhead and to inform interventions to reduce the incidence of human infection.

BACKGROUND

Salmonella bacteria are a common cause of human gastroenteritis worldwide. Only a small proportion of cases are diagnosed and reported, with the highest incidence of these being in young children.[1] *Salmonella* bacteria are also carried by a wide range of wild and domestic animals and can be excreted in their faeces.[1] There are more than 2,000 serotypes, with considerable global variation in prevalence and geographic distribution. Data from outbreak investigations of several common *Salmonella* serotypes have identified poultry, eggs, beef, milk, contaminated water, and raw fruit and vegetables, as vehicles of infection.[1–3]

Studies of risk factors for sporadic salmonellosis are uncommon. Risk factors for specific serotypes are usually identified by investigation of outbreaks; however, large case control studies of a common serotype in the United States and the United Kingdom, *Salmonella* Enteritidis, have demonstrated shell eggs (in particular the eggs of intensively-reared hens) to be the main risk factor for disease caused by this serotype in these countries.[4–5]

More than 200 cases of *Salmonella* Birkenhead disease are reported annually in Australia.[6–7] Over 90 per cent of these come from Queensland and New South Wales (NSW),[6–7] with the geographic distribution largely limited to southern Queensland and northern NSW (Figure 1). Between 1996 and 2002, 1,038 cases of *Salmonella* Birkenhead were notified in Queensland and 350 cases were notified in the Northern Rivers Area Health Service of NSW (data from the Queensland Notifiable Conditions Surveillance System and the NSW Notifiable Diseases Database). This represents seven per cent and 30 per cent of all *Salmonella* notifications in these regions respectively. An extensive review of the published literature failed to find any information regarding risk factors for infection due to *Salmonella* Birkenhead.

The specific geographical distribution of *Salmonella* Birkenhead, and the rarity of its isolation from animals involved in the food production chain,[8–12] suggest

FIGURE 1

***SALMONELLA* BIRKENHEAD NOTIFICATIONS BY STATISTICAL SUBDIVISION, QUEENSLAND AND NSW, 1996-2002**

Source: NSW Notifiable Diseases Database, NSW Health and Queensland Notifiable Conditions Surveillance System, Queensland Health

a potential environmental source. Environmental sources of *Salmonella* are thought to be important in the epidemiology of salmonellosis due to some serotypes.[13] Faecal contamination of the environment by native fauna or other animals could provide an opportunity for human exposure.

We conducted a case control study to explore risk factors for sporadic infection with *Salmonella* Birkenhead, in order to better understand the epidemiology of this serotype and to inform interventions to reduce the incidence of human infection. The choice of exposure factors to be investigated was influenced by our study hypothesis of an environmental source of infection; however, other potential sources of exposure were also included.

METHODS

Case and control selection

Cases were identified from notifications of *Salmonella* Birkenhead infection in Queensland and the Northern Rivers Area Health Service of NSW, through the routine notifiable disease reporting systems of each state, for the period October 2001 to December 2002. All the cases from Queensland were included, because Queensland has a centralised notification system simplifying the process for seeking ethics approval by requiring a single application to the Princess Alexandra Hospital Research and Ethics Committee. In NSW, where notifications are coordinated by each area health service, only the Northern Rivers Area Health Service, which had the greatest concentration of cases in NSW, was included. Ethics approval for this part of the study was obtained from the Northern Rivers Area Health Service Ethics Committee. To include all notifications from NSW would have required seeking ethical approval from multiple area health services for the addition of relatively few cases.

Notification of salmonellosis by pathology laboratories is mandatory under public health legislation in both Queensland and NSW. Cases were defined as residents of Queensland or the Northern Rivers Area Health Service of NSW, with a recent history of diarrhoea, and an infection with *Salmonella* Birkenhead confirmed by stool culture that was notified within the study period. To be eligible for the study, cases were required to meet the following criteria: they have adequate English skills; their infection was not acquired overseas; they were interviewed within 30 days of onset of diarrhoea; the onset of their diarrhoea was less than 10 days prior to specimen collection; they were not part of an identified outbreak; there was no other enteric pathogen isolated from the same faecal specimen; and no other member of their household had diarrhoea or was diagnosed with *Salmonella* infection in the four weeks prior to onset of illness.

Each case was matched with two controls using the age categories 0–4, 5–9, 10–19, 20–29, 30–59, and greater than 60 years. Controls were identified using a list of telephone numbers compiled from the electronic residential telephone directory. This list was randomly generated using a weighting system that provided a geographical distribution of telephone numbers approximating that of notifications. Potential controls were excluded if: they had suffered from diarrhoea in the four weeks prior to interview; they had inadequate English skills; or a member of their household had diarrhoea or was diagnosed with *Salmonella* infection in the four weeks prior to interview. Controls were interviewed as soon as possible and within a maximum of 30 days of the case's interview.

Questionnaire administration

Controls and cases were interviewed by telephone. All controls and the Queensland cases were interviewed by Queensland-based interviewers using a Computer Assisted Telephone Interviewing (CATI) system. The NSW cases were interviewed by one NSW-based public health officer using a paper-based version of the questionnaire. This design was selected due to concerns by the NSW researchers that it would be difficult to obtain ethical approval to have NSW cases interviewed by interviewers located interstate.

The questionnaire used was designed for the study. Conversion of the questionnaire to CATI format resulted in several minor differences relative to the paper-based questionnaire administered to NSW cases. Before the study commenced, the CATI interviewers received training that was tailored to the study, and during the study their interview technique was periodically monitored by supervisors. Questionnaire delivery and peer monitoring issues were discussed intensively with the interviewer of the NSW cases prior to the study, to support consistency of approach and minimise potential bias. A call-back protocol was established for the study, which standardised how many times a call was repeated if there was no answer.

For all cases and controls under the age of 15 years, or over 15 years but under 18 where the parent or guardian did not consent to a direct interview, information was obtained from the available parent or guardian who was most familiar with the case–control's diet and behaviour.

As well as demographic details and symptoms of illness, the questionnaire sought comprehensive information about a range of exposures including: contact with native fauna, farm animals and domestic pets; recreational activities involving potential contact with native animals or ingestion of water; and consumption of untreated water. The questionnaire explored food consumption and household food hygiene practices but did not include a detailed list of food items prepared and consumed. Exposure information for cases was sought for the seven days prior to the onset of illness. For controls, exposure information was sought for the seven days prior to interview. A copy of the questionnaire is available on request.

Sample size

The expected number of cases in the study region for a 12-month period, based on historical data, was approximately 170. Based on a case:control ratio of 1:2 with unmatched analysis, this number would enable the detection of an odds ratio of 2.0 at the five per cent significance level with 85 per cent power (assuming a 15 per cent exposure level among controls).

Data management and analysis

De-identified data were collected using EpiInfo version 6.04d.[14] Data quality was checked following entry into the database and prior to analysis. Data analysis was undertaken using SAS version 8.02.[15] We analysed the full range of exposure variables by calculating univariate odds ratios with 95 per cent confidence intervals (CIs). Statistical significance was assessed using the chi-square test for equal proportions. Multivariate analysis was conducted using stepwise logistic regression. All demographic and biologically plausible exposure variables with a univariate *P* value less than 0.2 were included in the initial model. A parsimonious model was arrived at by iterative removal of the variable with the highest *P* value, until only statistically significant parameters ($P < 0.05$) remained in the model. Stratified modelling by selected demographic variables was also undertaken to explore effect modification.

RESULTS

There were 217 cases of *Salmonella* Birkenhead infection notified during the study period. Of these, 75 were excluded due to: inadequate English skills (*n*=3); no history of diarrhoea (*n*=12); not interviewed within 30 days of onset of diarrhoea (*n*=25); onset of diarrhoea more than 10 days prior to specimen collection (*n*=7); part of an outbreak (*n*=2); infection acquired overseas (*n*=1); another enteric pathogen isolated from the same faecal specimen (*n*=10); household member with diarrhoea or diagnosed *Salmonella* infection in the four weeks prior to onset of illness (*n*=9); deceased (*n*=1), and for unspecified reasons (*n*=5).

Of the 142 eligible cases, 111 were enrolled, 30 from NSW and 81 from Queensland, a response rate of 78 per cent. Reasons for not responding were: physician not contactable or refused consent (*n*=10); refused to participate (*n*=1); no telephone number (*n*=9); and not contactable after six attempts (*n*=11). Diarrhoea was reported by all enrolled cases, fever by 80 per cent, vomiting by 51 per cent, and presence of blood in the stools by 29 per cent.

There were 429 controls identified. Of these, 99 were excluded due to: inadequate English skills (*n*=39); diarrhoea in the four weeks prior to interview (*n*=37); and another household member with diarrhoea or diagnosed *Salmonella* infection in the four weeks prior to onset of illness (*n*=23). Of the 330 eligible controls, 234 agreed to participate, 56 from NSW and 178 from Queensland, giving a response rate of 71 per cent.

Univariate analysis

Table 1 compares the demographic profile of the cases and controls. There was no significant difference between the groups for their age, sex, location of residence (urban or rural) and level of education. Results for selected exposure variables are presented in Table 2. The selection of these variables was informed by the literature and the study hypothesis (full results, stratified by state, are available on request). Fewer cases (69 per cent) than controls (78 per cent) reported eating non-home-cooked food (any food not prepared in their own home, excluding home-cooked meals consumed in another person's house: OR 0.6; 95 per cent CI 0.4–1.0). People who reported eating food not prepared in their own home were asked additional questions about the sources of this food. Specific sources that were associated with a significantly reduced risk included hamburger chains (OR 0.5; 95 per cent CI 0.3–0.9), pizza chains (OR 0.2; 95 per cent CI 0.1–0.6), fish and chip shops (OR 0.5; 95 per cent CI 0.2–1.0), bakeries (OR 0.4; 95 per cent CI 0.2–0.8), and sit-down restaurants (OR 0.4; 95 per cent CI 0.2–0.8). A significantly greater number of cases (17 per cent) than controls (nine per cent) reported not usually washing or peeling fruit or vegetables before eating raw (OR 2.1; 95 per cent CI 1.1–4.2). Of the environmental exposures, cases were more likely to have swum in a lake during the seven-day exposure period compared to controls (OR 3.7; 95 per cent CI 0.9–15.8).

Multivariate analysis

The final multiple logistic regression model included only the variables relating to fruit and vegetable washing–peeling and consumption of non-home-cooked food. Response categories for the non-home-cooked food variable included whether food was obtained from a fast food chicken chain. Cases were significantly more likely to: not usually wash or peel raw fruit or vegetables (OR 2.3; 95 per cent CI 1.1–4.7); have eaten only home-cooked food, that is, no reported consumption of non-home-cooked food (OR 1.9; 95 per cent CI 1.1–3.4); and to have eaten food from a fast food chicken source (OR 2.0; 95 per cent CI 1.0–4.0), compared to controls. As there were differences between the states in the prevalence of these variables, the results are presented stratified by state of residence (Table 3). In NSW, cases were significantly more likely to have eaten food from a fast food chicken source, and to not usually wash or peel raw fruit or vegetables, compared to controls. Eating home-cooked food only (that is, not reporting any consumption of non-home-cooked food) was the only significant risk factor for infection in Queensland residents.

The R-squared measure (a measure of the proportion of disease variation that is explained by a model) for the final model was 0.33 for NSW residents and 0.04 for

TABLE 1

DEMOGRAPHIC PROFILE OF *SALMONELLA* BIRKENHEAD CASES AND CONTROLS, QUEENSLAND AND NORTHERN NSW, OCTOBER 2001 TO DECEMBER 2002

Characteristic	Cases Number	Cases %	Controls Number	Controls %	Odds Ratio	(95% CI)#	*P* value
Sex	(*N*=111)		(*N*=233)				
Female	53	47.7	127	54.5	Reference Group		
Male	58	52.3	106	45.5	1.3	(0.8–2.1)	0.24
Age (Years)	(*N*=111)		(*N*=234)				
0–4	42	37.8	93	39.6	Reference Group		
5–9	7	6.3	14	6.0	1.1	(0.4–2.9)	0.84
10–19	16	14.4	32	13.6	1.1	(0.6–2.2)	0.78
20–29	4	3.6	8	3.4	1.1	(0.3–3.9)	0.72
30–59	29	26.1	58	24.7	1.1	(0.6–2.0)	0.73
60+	13	11.7	29	12.3	1.0	(0.5–2.1)	0.98
Place of residence							
Urban compared to rural	(*N*=109)		(*N*=233)				
Urban–Town	100	91.7	200	85.8	Reference Group		
Rural–Remote	9	8.3	33	14.2	0.5	(0.3–1.2)	0.12
Education level	(*N*=105)		(*N*=233)				
High (university degree)	29	28	83	36	Reference Group		
Medium	59	56	112	48	1.5	(0.9–2.6)	0.13
Low	17	16	38	16	1.3	(0.6–2.7)	0.45

CI = confidence interval

Source: Case Control Study of *Salmonella* Birkenhead infection in Queensland and northern New South Wales, Queensland Health and Northern Rivers Public Health Unit.

TABLE 2

UNIVARIATE ANALYSIS OF RISK FACTORS, SELECTED RESULTS, *SALMONELLA* BIRKENHEAD CASE-CONTROL STUDY, QUEENSLAND AND NORTHERN NSW, OCTOBER 2001 TO DECEMBER 2002

	Cases *N**	Cases %	Controls *N**	Controls %	Odds Ratio	(95% CI)#	*P* value
Animal contact							
Dog ownership	59/111	53	105/233	41	1.4	(0.9–2.2)	0.16
No pets kept	29/111	26	69/233	30	0.8	(0.5–1.4)	0.50
Lives on farm	9/110	8	22/229	10	0.8	(0.4–1.9)	0.67
Visited farm	18/107	17	33/234	14	1.2	(0.7–2.3)	0.51
Touched farm animals	5/111	5	12/234	5	1.0	(0.5–2.1)	0.67
Contact with manure	3/95	3	26/207	13	0.2	(0.1–0.8)	0.01
Touched native animals	1/108	1	13/232	6	0.2	(0.0–1.2)	0.04
Recreation in area with native animals	6/111	5	25/234	11	0.5	(0.2–1.2)	0.11
Water ingestion							
Drank any untreated water	21/105	20	38/231	16	1.3	(0.7–2.3)	0.43
Swam in lake	5/109	5	3/234	1	3.7	(0.9–15.8)	0.06
Food consumption							
Ate any non-home-cooked food	72/105	69	181/232	78	0.6	(0.4–1.0)	0.06
Ate food from fast food chicken chain	18/103	18	28/232	12	1.5	(0.8–2.9)	0.18
Don't usually wash or peel raw fruit–vegetables	18/106	17	20/227	9	2.1	(1.1–4.2)	0.03
Food preparation**							
Inadequate hand washing	43/94	46	76/209	36	1.5	(0.9–2.4)	0.12
Inadequate chopping board hygiene	26/85	31	58/185	31	1.0	(0.6–1.7)	0.90
Inadequate knife hygiene	29/92	32	73/202	36	0.8	(0.5–1.4)	0.44

* Missing and 'don't know' responses excluded.

** Asked only of respondents who reported preparing 2 or more evening meals per week and using meat, fish and poultry ingredients.

CI = confidence interval

Source: Case Control Study of *Salmonella* Birkenhead infection in Queensland and northern New South Wales, Queensland Health and Northern Rivers Public Health Unit.

TABLE 3

MULTIVARIATE ANALYSIS OF RISK FACTORS, *SALMONELLA* BIRKENHEAD CASE-CONTROL STUDY, QUEENSLAND AND NORTHERN NSW, OCTOBER 2001 TO DECEMBER 2002

	NSW			Queensland		
	Odds ratio	(95% CI)	*P*	Odds ratio	(95% CI)#	*P*
Don't usually wash–peel raw fruit–vegetables	8.5	(1.6–44.0)	0.01	1.5	(0.6–3.5)	0.35
Non-home-cooked food variable						
Ate non-home-cooked food (excluding from fast food chicken chain*)	Reference Group			Reference Group		
Ate only home-cooked food	1.4	(0.3–6.6)	0.28	2.1	(1.1–3.8)	0.01
Ate food from fast food chicken chain*	10.0	(2.7–36.7)	0.001	0.7	(0.2–1.9)	0.15

* Worded 'fast food chicken outlet' in questionnaire administered to NSW cases. # CI = confidence interval

Source: Case Control Study of *Salmonella* Birkenhead infection in Queensland and northern New South Wales, Queensland Health and Northern Rivers Public Health Unit.

Queensland residents. No significant change in findings occurred with addition of age group (six categories) to the model, and there was no significant effect modification by sex or rural–non-rural residence.

DISCUSSION

Our study identified three risk factors associated with sporadic *Salmonella* Birkenhead disease; eating food from a fast food chicken chain; not usually washing or peeling fruit and vegetables before eating raw; and consumption of only home-cooked food. Of these factors, eating food from a fast food chicken chain, and not usually washing or peeling fruit and vegetables before eating raw, were both associated with increased risk in NSW residents, while consumption of only home-cooked food was associated with an increased risk in Queensland residents. The questionnaire administered to NSW cases used the wording 'fast food chicken outlet', rather than 'fast food chicken chain', and it is possible that this wording may have led to differential classification of this study factor. However, there was no meaningful disparity between NSW and Queensland residents for the question relating to the fruit washing variable. There was potential for observer bias due to the different means of interviewing NSW cases. Although attempts were made to minimise this potential for bias, it remains an inherent weakness in the study design and illustrates some of the difficulties that may arise in conducting studies across different jurisdictions.

Consumption of takeaway chicken has been associated with an increased risk of sporadic salmonellosis in previous studies,[5] while consumption of raw fruit and vegetables have been implicated in previous salmonella outbreaks.[1] The increased risk observed in Queensland residents with consumption of only home-cooked food, while indirect and non-specific, could indicate an increased risk of acquisition of *Salmonella* Birkenhead from food prepared in the domestic kitchen. Domestic preparation of food has been considered a likely source for many cases of sporadic salmonellosis, although vehicles are seldom identified and domestic kitchen food handling risk factors are largely unknown.[2] While cross contamination from domestically prepared food has been identified as having an important role in *Salmonella* outbreaks,[16] its role in sporadic cases in unknown and our study failed to identify any food handling hygiene risk factors.[2] However, the lower than expected number of cases arising during the study period may have reduced the power of our study to detect significant risk factors. We did not investigate the consumption of specific food items prepared in the domestic setting.

Despite comprehensive investigation of potential environmental exposure pathways, we did not identify any significant environmental risk factors. Although we found an association with lake swimming, this was based on a small number of cases, had very wide confidence intervals and was not statistically significant in the multivariate analysis. Our results therefore fail to provide any evidence supporting our initial study hypothesis of a major role for environmental transmission in the epidemiology of *Salmonella* Birkenhead disease. It is possible that all relevant environmental risk factors may not have been fully captured in our questionnaire design. Contamination of a food source limited to this region is another potential explanation for the specific geographical distribution of this serotype.

Our final model explained 33 per cent of the variation in sporadic *Salmonella* Birkenhead disease in northern NSW residents and four per cent of the variation in Queensland residents. This indicates that major risk factors for *Salmonella* Birkenhead disease were not captured in our study. The reasons for the disparity between states remain unclear but could include different state patterns of contamination of food with *Salmonella* before or after the point of sale. The Queensland–NSW state border is physical as well as jurisdictional (the Border Ranges) and settlement patterns differ considerably between northern NSW and southern Queensland. However, the NSW model results should be interpreted with caution due to the small number of NSW cases and controls and the potential for observer bias from the different method used to interview

NSW cases. With consumption of food from fast food chicken sources identified as a possible risk factor, microbiological analysis of food samples from a random selection of these outlets could be considered. Further areas of exploration might include the investigation of distribution and supply patterns of chicken to fast food outlets in northern NSW and southern Queensland. Fruit and salad vegetables could plausibly be contaminated with *Salmonella* either before or after the point of sale. As the variable 'not usually washing or peeling fruit and vegetables before eating raw' could be a marker for consumption of locally grown fruit and vegetables, any future investigation should consider the consumption of local produce. If further research corroborates these findings, public health education encouraging the washing or peeling of all fruit or vegetables to be eaten raw may be of benefit.

CONCLUSION

This report illustrates some of the difficulties involved in studies of unlinked sporadic cases of salmonellosis, which often fail to fully explain mechanisms of transmission. Despite the largely negative findings of our study, particularly in relation to environmental factors, we did identify three risk factors that were associated with sporadic *Salmonella* Birkenhead disease. While these associations have been demonstrated in previous studies of salmonellosis caused by other serotypes, the associations identified in our study could have arisen due to chance or methodological bias.

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