

OUTBREAK REPORT

Investigation of a *Salmonella* Saintpaul Outbreak in the Auckland and Waikato Regions.

Pat Neuwelt, Public Health Registrar

Craig Thornley and Greg Simmons, Medical Officers of Health

Jasmine Mohiuddin, Technical Officer
AUCKLAND REGIONAL PUBLIC HEALTH SERVICE

With the assistance of Bruce Butters, Health Protection Officer, Mid-Central
Public Health Service, Whanganui.

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INTRODUCTION

The incidence of notified cases of salmonellosis in New Zealand has declined in the past five years, from a rate of 48.1 per 100,000 population in 2000 (ESR 2002) to a rate of 28.9 in 2004 (ESR 2004). The greatest disease burden of reported salmonellosis is among children under ten years of age. In 2004 the rate among children under ten years ranged from 122.6 (infants) to 37.7 (5-9 years) (ESR 2004). These figures underestimate the actual burden of the disease in the population.

Salmonella enterica serotype Saintpaul (*S. Saintpaul*) is a rare cause of human salmonella infection in New Zealand, identified as the infecting organism in 1.7% of reported salmonellosis cases in 2003 and 2.7% in 2004 (ESR 2005). It is found in a wide range of animals, though reptiles most commonly (Taylor et al 2000), and isolates are frequently found in New Zealand geckos (Nicol 2005). Most reported *S. Saintpaul* cases occur in the South Island. No cases were reported from the Auckland region during 2003 and 2004.

During the months of July and August 2005, sixteen cases of *S. Saintpaul* infection were notified to the Auckland Regional Public Health Service, and enquiries revealed that *S. Saintpaul* cases had also been reported in Waikato. Preliminary interviews suggested a high frequency of raw carrot consumption in the three days prior to illness. This paper outlines the epidemiology and case-control investigation of this *S. Saintpaul* outbreak.

METHODS

Epidemiological Investigation

A routine salmonellosis questionnaire was initially completed by Health Protection Officers over the telephone with each notified case, or parent of a case. Hypotheses about the source of the outbreak were developed from the results of these interviews. A case-control study was performed to test these hypotheses. Study cases were defined as persons living in the Auckland or Waikato regions who were notified to a public health service as having developed diarrhea (≥ 3 loose stools in a 24-hour period) after 1 April, 2005, with faecal specimen positive for *S. Saintpaul*.

Each case was matched with three controls, found by calling sequential telephone numbers commencing with the case's residential telephone number. The person in the control household with the next birthday was eligible to participate in the study. If the eligible person was a child (<15 years), the telephone questionnaire was completed on his/her behalf by a parent or guardian. Controls were ineligible if they met either of the following criteria:

1. They had suffered from gastroenteritis (defined as ≥ 3 loose stools in any one 24-hour period) since 15 March, 2005.
2. They had insufficient English-language ability to complete the questionnaire.

Telephone numbers were called three times, unless disconnected or a fax or business line, before moving on to the next number. At least one of the three calls was made after 1800 hours. Cases and controls were not matched other than by telephone number, a proxy for locality.

Cases and controls were interviewed using the same questionnaire. The questionnaire included demographic data and specific questions about the consumption of meat and vegetables, either during the three-day period prior to onset of illness (cases) or during the three-day period prior to interview (controls). Data were analysed using EpiInfo version 3.3.2 (Dean et al 2000). Univariate matched and unmatched odds ratios (OR) and 95% confidence intervals (CI) were calculated for the exposures. The demographic distribution of the case and control groups were compared using chi-square tests (for differences in age, gender or ethnicity distribution) and an ANOVA test (for differences in mean age between these groups). Logistic regression analysis was performed to control for confounding. P-values were subject to Yates' correction, except where a cell contained a value less than five, in which case the Fisher exact test was applied.

Traceback and Environmental Investigation

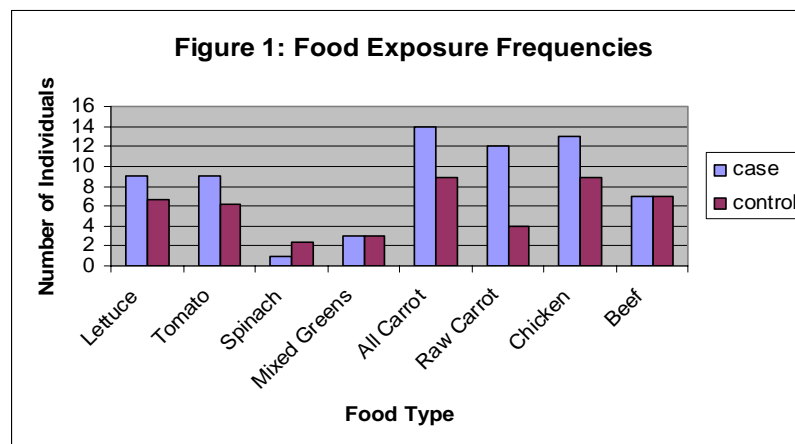
Traceback information was collected from cases who could remember the brand, place and date of purchase of the carrots they had consumed within three days of becoming ill.

Retailers were approached for the names of distributors and packers. The names of producers were collected from pack houses. Purchase dates, and presumed purchase dates based on case onset dates, were used in traceback.

Washings for culture were taken from Auckland pack houses, as well as from water supply hoses on the carrot washing machinery at the implicated farms. Multiple soil specimens were collected from the farms for culture.

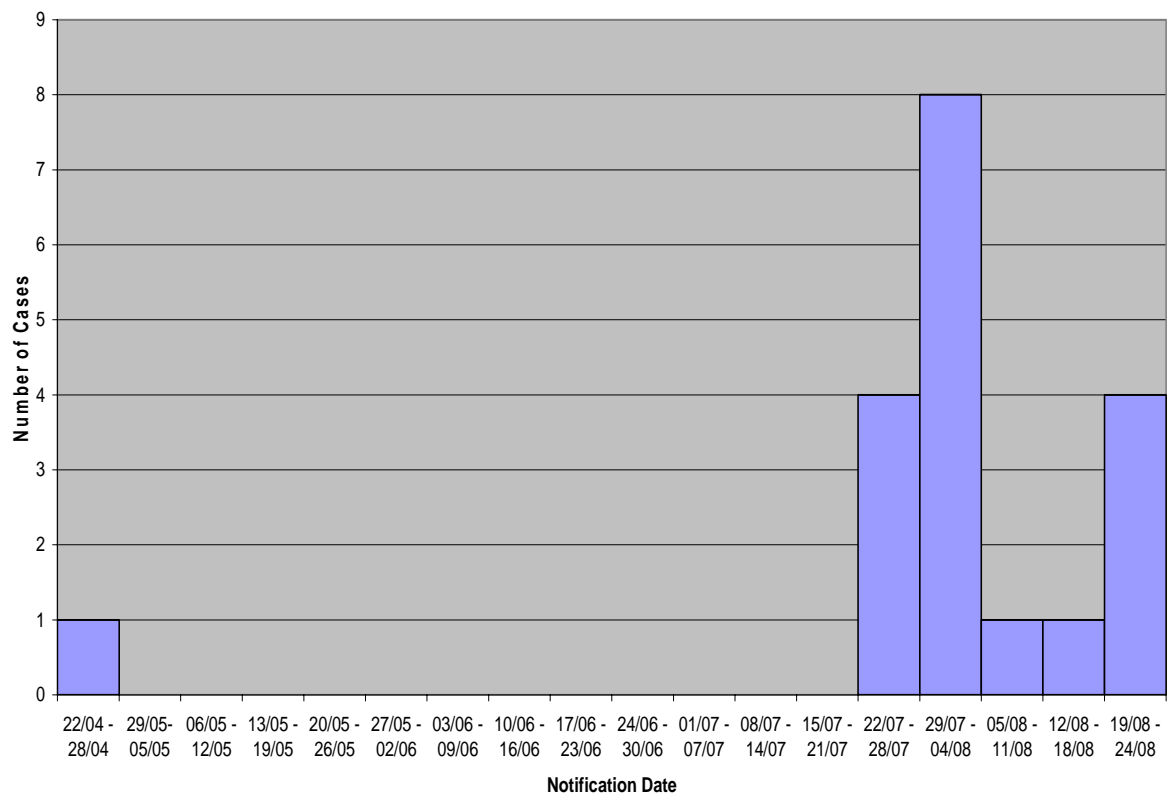
RESULTS

Preliminary interviews with the initial cohort of 13 cases suggested that carrots were the most frequently consumed vegetable (85%) during the three days prior to illness onset, with lettuce rating second (69%). Chicken was most frequently consumed meat during that same period for cases (71%) with beef rating second (64%) (see Figure 1).



A total of 19 cases, reported between 17 April and 4 August 2005, met the study case definition; 17 in Auckland (see Figure 2) and two from Waikato. All of these participated in the study. Cases ranged in age from one to sixty-three years, with most being aged ten years or under.

Figure 2: Epidemic Curve of Notified Salmonella Saintpaul Cases, Auckland Region



Full clinical information was available for 16 of the 19 cases. Symptoms ranged from moderate to severe, and included stomach cramps, nausea and vomiting, diarrhea (sometimes bloody), fever and headaches (see Table 1). The illness lasted for three to ten days, with a median duration of 7 days. Hospitalisation data was available for 14 of the 19 cases. Two cases, with severe symptoms, were hospitalised; one was treated in the emergency department then discharged, and the other was admitted with a query of appendicitis, prior to culture becoming available.

Table 1: Frequency of Symptoms among Cases

<i>Symptom</i>	<i>Number of cases (%), n=16</i>
Diarrhea	16 (100)
Bloody diarrhea	02 (13)
Stomach cramps	12 (75)
Fever	09 (56)
Nausea	06 (38)
Vomiting	03 (19)
Headache	03 (19)
Lethargy/malaise	02 (13)

It required 287 telephone calls to identify 57 participating controls. There were 27 potential participants deemed ineligible (either a non-English speaker, a business, or excluded on the basis that they had had diarrhea since 15 March/05). A further 40 people contacted by telephone declined to participate.

Fifty-seven percent of cases were children (aged <15 years), a significantly greater proportion than among controls (12.3%, chi-square=13.98, 95% CI 2.55-39.91, p<0.01). The mean age of cases (7 years) was also significantly less than that of controls (35 years, ANOVA p-value <.01). There was no significant difference between the two study populations by gender (chi-square=1.45, CI 0.14-1.48, p=0.23) or ethnicity (European or not, chi-square=1.52, CI 0.57-105.96, p=0.22) (see Table 2).

Consumption of raw carrots was the only exposure with a significant univariate association with increased risk of illness (see Table 3). During the exposure period of interest, 12 cases (63.2%) and 17 controls (29.8%) had consumed raw carrots (unmatched odds ratio [OR] 4.0, CI 1.35-12.01, p=0.02; matched OR 7.3, CI 1.8-30.6, p=0.01). (Note: Some data on vegetable consumption were missing for three cases.)

Logistic regression analysis was performed to control for age (<15 years or not). It revealed a less significant adjusted odds ratio for raw carrot consumption (OR 2.86, CI 0.66-12.3, p=0.16). No significant association was demonstrated between having contracted salmonellosis and having reported scrubbing (OR 2.84, 0.80-10.17, p=0.12) or peeling (OR 2.75, 0.83-9.23, p=0.11) carrots before consuming them raw.

Table 2: Demographic Characteristics of Cases and Controls

	<i>Cases (n=19)</i>		<i>Controls (n=57)</i>		Chi-Square
	Number	%	Number	%	
<i>Median age in years (range)</i>	7 (1-63)		35 (2-85)		
<i><15 years</i>	11	57.9	7	12.3	13.93 (p<0.01)
<i>>15 years</i>	8	42.1	50	87.7	
<i>Sex</i>					
<i>Female</i>	8	42.1	35	61.4	1.45 (p=0.23)
<i>Male</i>	11	57.9	22	38.6	
<i>Ethnicity*</i>					
<i>European</i>	18	94.7	45	78.9	1.52 (p=0.22)
<i>Maori</i>	1	5.3	3	5.3	
<i>Pacific</i>	0		1	1.8	
<i>Asian</i>	1	5.3	4**	7.0	
<i>Other</i>	0		3***	5.3	
<i>Not identified</i>	0		1		

*1 case self-identified Maori & European, therefore cases total=20 and percentage>100.

** 3 Korean, 1 Chinese *** 1 Indian, 1 Australian, 1 "Muslim"

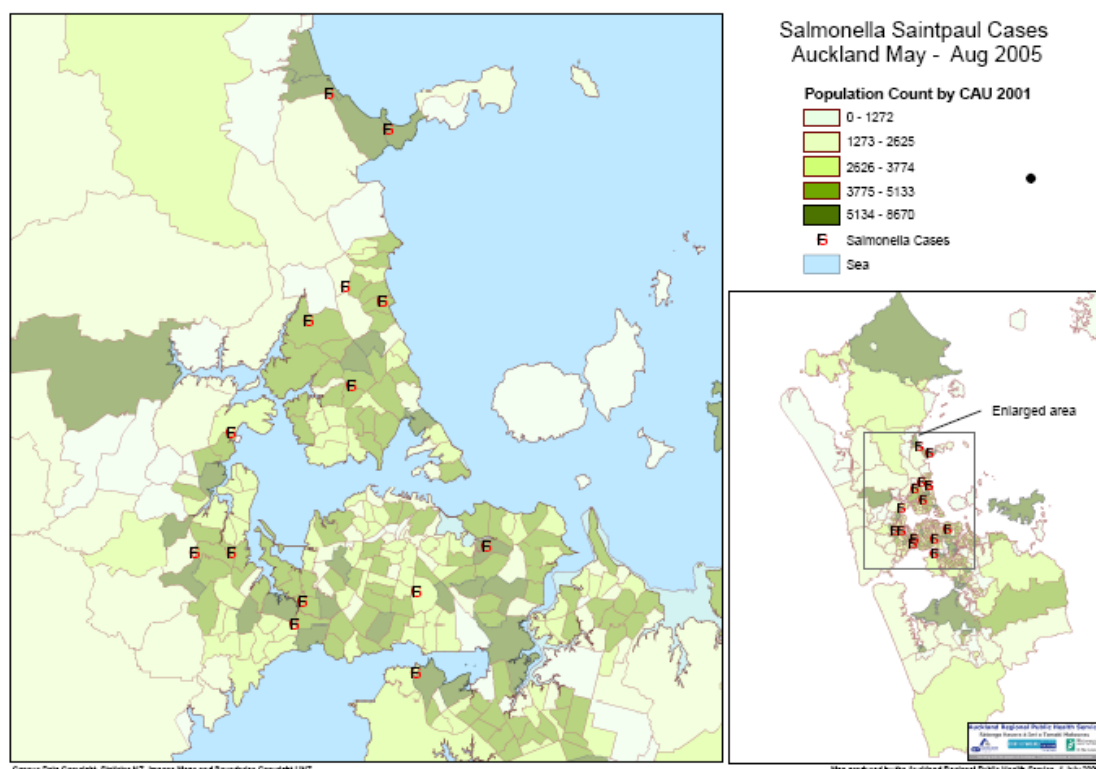
Table 3: Frequency of Selected Exposures among Cases and Controls

	<i>Cases (n=19)</i>	<i>Controls (n=57)</i>	<i>Univariate Odds Ratio (95% CI)</i>	<i>P-value</i>
Beef	7 (36.8%)	30 (52.6%)	0.65 (0.18-2.27)	0.63
Chicken	13 (68.4%)	37 (64.9%)	1.17 (0.39-3.55)	1.00
Salad greens (lettuce, spinach, etc)	11 (57.9%)	38 (66.7%)	0.69 (0.24-2.00)	0.68
Tomatoes (raw)	9 (47.4%)	31 (54.4%)	0.84 (0.26-2.74)	0.96
All carrots	14 (73.7%)	33 (57.9%)	2.03 (0.65-6.42)	0.34
Raw carrots	12 (63.2%)	17 (29.8%)	4.03 (1.35-12.01)	0.02

Traceback and Environmental Investigation

Auckland cases lived in all parts of the region (see Figure 3). They had purchased carrots from multiple different retailers in the region. The Waikato cases were in two different small towns in the region.

Figure 3: The first 15 *Salmonella* Saintpaul cases in Auckland by home address*



*Note: two of the cases were in the same household.

Three Auckland packing houses were identified as having supplied carrots to the implicated retailers. These were visited by a Health Protection Officer, and food safety assessments were carried out to identify the potential hazards and assess the adequacy of the critical control measures being carried out at the premises. Equipment was tested for presence of *Salmonella* at each site, and all cultures were reported negative. There were no reports of staff illness with gastroenteritis during the period of interest. Of note was the fact that none of the packing houses were washing the carrots on-site prior to packaging.

Four farms were identified as the sources of carrots consumed by the majority of cases. Each of these farms was located in the same region. Site investigations were performed on the implicated farms by a Health Protection Officer from the nearest regional public health service. As with the packing houses, there were no reports of staff illness with gastroenteritis during the period of interest. None of the farms used organic fertilisers. Three of the four farms used stream water to rinse the carrots after harvesting and pipe water samples were taken from each of these (see Figures 4-6). Specimens revealed a high coliform count (total coliform count range 460 to >2400, *E.coli* count range 9.8 to 88/100 mL) but no *Salmonella* growth. One farm was on municipal water, which was not tested. Of potential relevance was the fact that carrots were being rinsed outdoors, using tumblers which would remove the tops off the carrots, and which could potentially damage the surface of the carrots. Unfortunately,

these tumblers were not sampled for *Salmonella* at the time of the farm visits. Soil samples were taken from fields from which produce was harvested during the period of interest, and subsequently tested negative for *Salmonella*. Carrots from the implicated farms were not sampled, as there was a significant delay between the end of the outbreak and the farm visits. Attempts to retrieve seed samples from the farms in question were also unsuccessful. Further, rainfall data was requested from the National Institute of Water and Atmospheric Research for the immediate area of the farms, but due to equipment failure during the month of July, the data was not readily available.

Figure 4: Farm #1 - Carrots arrive in big green hopper and are rinsed with stream water in a tumbler outdoors.



Figure 5: Farm #2 – Carrot tops are removed in a large tumbler outdoors, while being sprayed with stream water.



Figure 6: Farm #3 – Carrots rinsed outdoors with stream water before being transported to pack-house for bagging.



DISCUSSION

This study demonstrated a possible association between consumption of raw carrots and salmonellosis due to *S. Saintpaul* infection. Consistent with the national trends for human salmonellosis, this outbreak affected many more children than adults.

There have been no reports in New Zealand of *Salmonella* outbreaks due to contaminated produce; however there have been a number of such reports overseas. In the USA, outbreaks have involved tomatoes (Hedberg et al 1999; Cummings et al 2001) and alfalfa sprouts (Gill 2003). Lettuce has also been implicated as a source of *Salmonella* exposure in the UK (Horby et al 2003) and Australia (Stafford et al 2002). None of the overseas outbreaks involved the Saintpaul serotype. The critical control-point failures predisposing people to *Salmonella*-contaminated produce have been difficult to ascertain. Most often, the traceback investigation has led to packing houses and farms, and appears to be related to issues with water quality control, as in this study. Manure is recognised as an obvious source of pathogens in soil; however, runoff water from fields or bush, and irrigation water containing manure may be important sources of produce contamination with enteric pathogens.

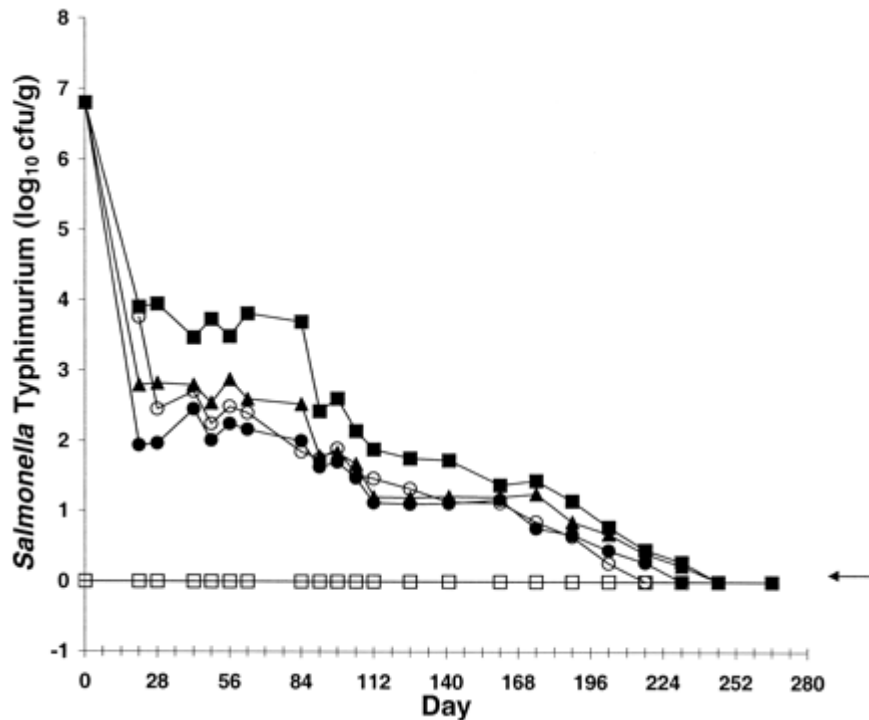
Studies in controlled environments, both in the USA and New Zealand, have shed some light on the complexity of produce contamination by manure. It has been demonstrated that contaminated manure compost and contaminated irrigation water can cause the contamination of not only soil, but vegetables as well (Islam et al 2004a, Islam et al 2004b). In one study, *Salmonella* typhimurium was found to persist in soil, which had been contaminated with poultry or cow manure, for over 200 days, and was detected on radishes and carrots 84 and 203 days, respectively, after sowing (Islam et al 2004a). Figure 7 below, taken from the Islam et al. study, highlights the finding that *Salmonella* enterica can persist on carrots grown in compost-contaminated soil for over 6 months.

Further research has demonstrated that bacterial contamination of vegetables can go beyond the surface to core plant tissues. A New Zealand study found that both *E. coli* and *Salmonella* typhimurium could be detected not just on the surface of, but in the roots and shoots of lettuce plants grown from seed in soil contaminated with those bacteria (Horswell 2005). The study also found that surface contamination of lettuce seedlings with the bacteria led to the internalisation of both pathogens.

Even in large outbreaks of salmonellosis related to contaminated produce in North America and the UK, the original source of contamination has seldom been traced (Cummings et al 2001; Hedberg et al 1999; Horby et al 2003). As in this study, the traceback investigation is an inexact process which relies heavily on the cooperation of food retailers, packers and producers.

Of interest is one waterborne outbreak of *S. Saintpaul* in Queensland during March 1999 (Taylor et al 2000). The source of the infection was found to be contaminated drinking water. The outbreak investigation concluded that unusually heavy rainfall had led to the excreta of mice and frogs being washed into the water collection system at the implicated construction site. A similar link between heavy rainfall and contaminated runoff into stream water, used to wash carrots, might exist in this study. *S. Saintpaul* is frequently isolated from New Zealand geckos (Nicol 2005), and the water catchment area for the streams implicated in this study includes extensive bush and alpine scrub, the natural habitat of geckos.

Figure 7: Survival of *S. enterica* serovar Typhimurium in inoculated-compost-amended or inoculated-water-irrigated soil samples from fields used for growing carrots.



Treatments included no compost (□), poultry manure compost (■), dairy cattle manure compost (▲), alkaline-pH-stabilized dairy cattle manure compost (●), and contaminated irrigation water (○). The arrow indicates that the organism was not detectable by enrichment culture. (Islam et al 2004a)

The power of this case-control investigation was limited by the small number of cases. While the association demonstrated between carrots and salmonellosis was not statistically significant, the association is both consistent with overseas experience regarding the contamination of produce and it is biologically plausible, given the research evidence discussed previously. The traceback investigation revealed that the carrots in question were rinsed at the farms after harvesting to remove soil, using stream water contaminated with *E. coli* and unsuitable for drinking. The carrots were not routinely washed again before being sold to consumers. It is plausible, then, that there might have been a sporadic contamination of the stream water with *S. Saintpaul*, related to heavy rainfall and contaminated runoff from bush or alpine scrub upstream. The carrots harvested at that time, with surface damage due to the mechanical process used to rinse and remove the tops, might then have had significant surface contamination with *S. Saintpaul*. Although the study did not demonstrate that cases were less likely to wash or peel carrots before consumption raw, controls were interviewed in late August, well after the last notification of *S. Saintpaul* salmonellosis in Auckland.

After controlling for age, the association between raw carrot consumption and *S. Saintpaul* salmonellosis remained elevated but lost statistical significance, indicating that the association was at least partly confounded by age. It can also be argued that young age was a potential effect modifier, if in fact children are more likely to eat raw carrots than adults. Carrot consumption appears to be comparable between New Zealand children and adults. According to the National Nutrition Survey, 83% of New Zealand adults (>15 years) report consuming carrots at least once per week, and six percent consume them once per day

(Ministry of Health 1999). Children aged 5-14 years are slightly less likely to eat carrots overall (79%), yet 6.3% report eating them once per day (Ministry of Health 2003). No distinction is made in either survey between raw and cooked carrots.

The risk of contracting salmonellosis may be associated with the consumption of inadequately prepared raw vegetables. If children eat raw carrots more than adults, they are more likely to be at risk from exposure to pathogens on carrots.

The findings of this investigation do point towards produce as a potential source of human salmonellosis in New Zealand, and will be provided to the New Zealand Food Safety Authority for consideration of two issues raised by this outbreak. First, water quality standards for washing fresh produce need to be reviewed. Second, produce which is more likely to be consumed raw, such as salad vegetables, should be labelled with a recommendation that they are washed well and, if appropriate, peeled, before being consumed.

This study failed to confirm carrots as the source of infection, due to a whole range of factors. However, incident cases of *Salmonella* Saintpaul infection should be interviewed with a view to obtaining a careful history about vegetable consumption. Further, given that the water used to rinse carrots may contain significant levels of *E. coli*, and intermittently *Salmonella*, consumers would be wise to scrub, peel and thoroughly rinse carrots before eating them raw. Further local studies are needed to clarify the full significance of this potential public health issue for New Zealand.

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Appendix 1: Salmonella Saintpaul outbreak case questionnaire

AK2005158

Case Episurv. Number: 2005AKO _____ Telephone
number: _____

Name of Case:.....

**All of
the
follow
ing**

questions refer to the period of three days before illness.

Date of onset of symptoms:...../...../.....

Did you (or the case) consume any of the following in the THREE days before your illness?

(Circle one of Y=yes, N=no, U=unsure)

1. MEAT

a. Would you have eaten chicken?..... Y N U

b. Would you have eaten beef?..... Y N U

2. VEGETABLES

a. Would you have eaten salad greens including lettuce, spinach or other green leafed vegetables?

..... Y N U

If YES: What type?

Did you eat lettuce?..... Y N U

(tick box)

- Head
 - Loose leaf packaged
 - Loose leaf bin
 - Hydroponic

Did you eat spinach?..... Y N U

- Bunch
- Loose leaf packaged
- Loose leaf bin

Did you eat mixed greens..... Y N U

- Loose leaf packaged
- Loose leaf bin

When was it purchased?.....

Where was it
purchased?.....

.....

Details of brand or packaging including
colour.....

.....
b. In the three days before illness did you eat uncooked tomatoes (including cherry)?

..... Y N U

If YES,

When was it
purchased?.....

Where was it
purchased?.....

.....
.....

How were they purchased?

- Pre-packaged or bagged tomatoes
- Loose tomatoes

Details of brand or packaging including
colour.....

.....
.....

How were they prepared (sliced/whole)?

.....

c. In the three days before illness did you eat carrots.....Y N U

If YES,

How were the carrots purchased?

- Packaged baby carrots
- Pre-bagged unpeeled carrots
- Loose unpeeled carrots

When were the carrots purchased?

.....

Where were the carrots purchased?

.....

Do you remember a brand name or packaging details?

.....

.

How were they prepared for eating (scrubbed, peeled, etc)?

.....

.....

.....

.....

How were the carrots eaten?

- Raw
- Cooked

If COOKED, How were the carrots cooked (steamed, boiled, microwaved)?

.....
.....

d. Did you eat sandwiches (including salad filling) Y N U

If YES, what were the fillings?

- Lettuce
- Capsicum
- Cucumber
- Carrot
- Sprouts
- Tomato
- Celery
- Mushrooms

Was it purchased pre-made?..... Y N U

When was the sandwich
purchased?.....

Where was it
purchased?.....

Thank you for your help and continued cooperation with this investigation.

Would you like to receive a summary of the outbreak report? Y N

If yes.....we will post it once the investigation is complete.

Do you have any questions regarding the investigation? If you think of some later please call 623 4600 and ask for Dr Craig Thornley or Dr Pat Neuwelt

Date questionnaire completed...../...../..... Interviewer:.....

Appendix 2: Salmonella Saintpaul outbreak control questionnaire

AK2005158

control letter (a,b,c)

Case Episurv. Number: 2005AKO _____ Control Telephone
number: _____

Name of Control:..... Suburb:.....
--

**All of
the
follow
ing**

questions relate to foods eaten for the three days before today.

Did you consume any of the following in the THREE days before today?

(Circle one of Y=yes, N=no, U=unsure)

3. MEAT

b. Chicken Y N U

b. Beef..... Y N U

4. VEGETABLES

e. **Would you have eaten salad greens including lettuce, spinach or other green leafed vegetables?**

..... Y N U

If YES:

Did you eat lettuce?..... Y N U

(tick box)

- Head
 - Loose leaf packaged
 - Loose leaf bin
 - Hydroponic

Did you eat spinach?..... Y N U

- Bunch
 - Loose leaf packaged
 - Loose leaf bin

Did you eat mixed greens..... Y N U

- Loose leaf packaged
- Loose leaf bin

f. In the three days before illness did you eat uncooked tomatoes (including cherry)?

..... Y N U

If YES,

How were they purchased?

- Pre-packaged or bagged tomatoes
- Loose tomatoes

How were they prepared (sliced/whole)?

.....

g. In the three days before illness did you eat carrots.....Y N U

If YES,

How were the carrots purchased?

- Packaged baby carrots
- Pre-bagged unpeeled carrots
- Loose unpeeled carrots
-

How were they prepared for eating (scrubbed, peeled, etc)?

.....
.....

How were the carrots eaten?

- Raw
- Cooked

If COOKED, How were the carrots cooked (steamed, boiled, microwaved)?

.....
.....

h. Did you eat sandwiches (including salad filling)Y N U

If YES, what were the fillings?

- Lettuce
- Capsicum
- Cucumber
- Carrot
- Sprouts
- Tomato
- Celery
- Mushrooms

Was it purchased pre-made?..... Y N U

Thank you for your help with this investigation.

Do you have any questions regarding the investigation? If you think of some later please call 623 4600 and ask for Dr Craig Thornley or Dr Pat Neuwelt

Date questionnaire completed...../...../..... Interviewer:.....