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Fresh garlic: a possible vehicle for *Salmonella* Virchow

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SUMMARY

A sustained increase in *Salmonella enterica* serovar Virchow notifications in South Eastern Australia between September 1997 and May 1998 instigated a case-control study and environmental investigations. Cases were defined as having locally acquired culture-confirmed *S.* Virchow phage-type 8 infection and diarrhoeal disease. Matched controls were selected by progressive digit dialling based on cases' telephone numbers. An exposure and food history questionnaire was administered by telephone. Phage typing and pulse field gel electrophoresis were performed on case and environmental isolates. Thirty-two notifications of *S.* Virchow infection met the case definition, 37% reported bloody diarrhoea and *S.* Virchow was isolated from blood in 13% of cases. Twelve patients were admitted to hospital and one died. Fresh garlic (OR 4·1, 95% CI 1·3–12·8) and semi-dried tomatoes (OR 12·6, 95% CI 1·5–103·1) were associated with these cases. The associations remained significant after adjusting for sex and age. *S.* Virchow (PT 8) was cultured from two brands of semi-dried tomatoes associated with cases in two different states. We provide sufficient evidence for semi-dried tomatoes and fresh garlic to be considered as potential risk foods in future *Salmonella* outbreak investigations.

INTRODUCTION

Salmonella enterica serovar Virchow (*S.* Virchow) is recognized as one of the more common, invasive *Salmonella* serotypes [1–7] and accounted for 46% of septicaemia cases caused by *Salmonella* species in a study of hospital laboratory records in Queensland [4]. Any increase in notification of this serotype in Australia is reason for concern.

Notifications of *S.* Virchow infection are usually uncommon in the southern states of Australia. The 10-year (1988–1997) annual mean for the number of case notifications in New South Wales was 25 [8]. In the first quarter of 1998, 78 cases of *S.* Virchow infection were notified in New South Wales compared to 24 cases in the same period in 1997 [9]. Moreover,

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S. Virchow accounted for 2·6% of salmonella notifications in 1997 [10] and 16% in January–March 1998 [8]. Approximately 90% of the 1998 first-quarter isolates were phage type 8 (PT 8).

We describe the results of a case-control study and environmental investigation into the source and vehicle(s) responsible for this outbreak.

METHODS

A population-based case-control study was conducted prospectively over 1 year, from 15 April 1998 to 14 April 1999, to determine the outbreak vehicle(s) and source. Cases were defined as individuals residing in New South Wales, Victoria, South Australia or the Australian Capital Territory who had locally acquired culture-confirmed *S*. Virchow (PT 8) infection with a diarrhoeal illness ($\geqslant$3 loose stools in 24 h) with a clearly defined onset. Individuals exposed to other cases of diarrhoeal illness in the month prior to illness were excluded ($n=10$), as were children less than 1 year of age ($n=2$) as they were more likely to be secondary cases.

For each case, two controls were selected from households identified using progressive digit dialling based on the case's home telephone number. This method of selection provided a loose geographical match between cases and controls that was deemed necessary as early case reports were concentrated in a semi-rural location in New South Wales. To facilitate rapid recruitment of controls and to minimize the risk of overmatching, the cases and controls were only matched on a geographical basis. Sex and age were adjusted for in logistic regression analyses.

Recruitment telephone calls were made on weekdays, evenings and weekends to minimize bias against households where all members have daytime jobs or are in school. A minimum of five attempts was made to each potential control telephone number, and at least two of the five attempts were made outside normal daytime working hours. Controls were randomly selected within households as the person with the next birthday, but were excluded if they were less than 1 year of age and/or had diarrhoeal illness in the month prior to interview.

Information on food and other exposures for the 5 days prior to illness for cases and prior to interview for controls was collected using a questionnaire administered by telephone. The New South Wales Health Department's salmonellosis questionnaire was modified to include more detail on specific foods that had been associated with *S*. Virchow in prior reports: poultry and eggs, rare or raw meat, unpasteurized milk products and macadamia nuts [11, 12].

Details were also collected on method of food preparation, brand, place of purchase and date consumed, as well as food-handling and preparation practices, and environmental contact with potential sources of salmonella. Semi-dried tomatoes, and the ingredients used in their manufacture, were added to the questionnaire after we received a report from a salmonellosis case investigation in another state where *S*. Virchow had been isolated from an open container of semi-dried tomatoes.

Environmental investigations included tracing food consumed by cases back to commercial outlets, tracing commercial products to the manufacturer and investigating and tracing ingredients upstream to distributors and producers. Inspections of commercial premises, including food production techniques and preparation sites, were also carried out. Commercial and domestically prepared food were sampled and cultured for *Salmonella* species for which we had epidemiological evidence implicating it as a risk food in this outbreak, or linking a specific product or batch directly to cases.

Data were analysed using matched odds ratios (ORs) and adjusted matched ORs using logistic regression (Stata version 7).

Pulsed field gel electrophoresis (PFGE)

All human and environmental *S*. Virchow isolates were phage typed and further sub-typed using pulsed field gel electrophoresis (PFGE). PFGE was essentially performed according to Maslow et al. [13]. Restriction endonuclease *Xba*I was used to digest salmonella genomic DNA. Either Biorad CHEF DR-II or CHEF DR-III apparatus was used to separate restriction fragments. After electrophoresis, the gel was stained in ethidium bromide for 30 min and photographed under UV light illumination. Dendrograms were generated by the GelCompar version 4.1 program (Applied Maths, Kortrijk, Belgium) using the unweighted pair group method (UPGMA) for clustering on a matrix based on the pair-wise comparisons of the Dice coefficient.

RESULTS

The case-control study included cases with onset of illness between 15 April 1998 and 15 April 1999.

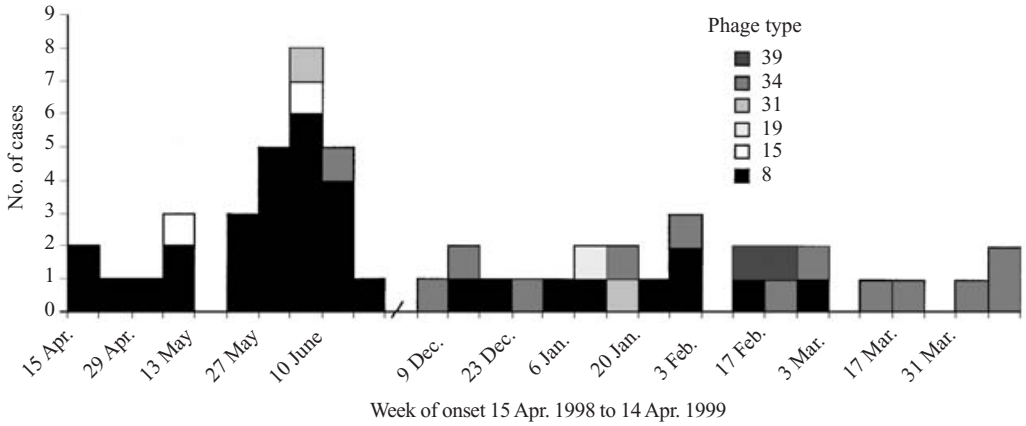

Fig. 1. Epidemic curve: *Salmonella* Virchow notifications by month of onset (15 April 1998 to 15 April 1999).

Eighty-five cases of *S*. Virchow infection were notified during the study period and 54 cases were deemed eligible and all agreed to participate in the investigation. The most common reasons for exclusion of cases were: exposure to other people with diarrhoeal illness ($n=10$), overseas or interstate travel during incubation period ($n=6$), or a lack of definite onset date and/or diarrhoeal illness ($n=6$).

Thirty-two of those initially recruited and interviewed were subsequently typed as having PT 8 infection and met the case definition. The remaining isolates were identified as PT 15, 19, 31, 34 and 39 (Fig. 1) and were excluded from analyses. For controls, 106 (69%) of the potential controls identified by random digit dialling participated in the study, and 64 were matched to PT 8 cases and so were retained for analyses. Females were over-represented among cases (19F:13M) compared to controls (26F:38M) and cases had a median age of 22 years (range 1–75 years) compared to 42 years (1–78 years) for controls. Nine of the 32 PT 8 cases were aged 11 years or less.

The mean delay from onset to interview for cases was 19 days (range 9–47 days). The time interval between the date of onset of illness for a case and the date of interview for their matched controls ranged from 13 to 80 days (median 41 days).

In addition to diarrhoea, illness was characterized by abdominal pain (90%), fever (87%), nausea (77%), joint pain (59%) and vomiting (48%). *S*. Virchow was isolated from blood in four cases (13%). One person died and 12 (39%) were hospitalized in the 31 cases where this information was recorded. Fourteen of the 16 employed cases (88%) missed work (median 5 days). As cases were identified on the basis of laboratory tests, all cases had visited a medical practitioner at least once.

When phage typing commenced in July 1998, 86% of case isolates were found to be PT 8 (Fig. 1) We submitted a panel of *S*. Virchow isolates, which included strains from this and previous investigations, to PFGE with the enzyme *Xba*I. We were not able to discriminate further between isolates for our PT 8 cases using PFGE, though they could be distinguished from PT 8 isolates from previous investigations. A total of 50 strains were subjected to PFGE, including both outbreak and non-outbreak strains. Group A comprised 24 strains, 20 of these were PT 8, the remainder were PT 34 (2 strains), PT 17 (1 strain) and 1 strain of unknown phage type. All human and environmental isolates from this case control study were identified as PFGE group A, with 95% or better similarity (Fig. 2). The remaining 26 non-group A strains (including group A2) comprised 15 PT 8 strains (43%) and members of PT 2, 11, 16, 17, 31, 33, 34 and 39 strains.

We analysed frequencies of exposure for PT 8 cases and controls (Table 1). Logistic regression was carried out to adjust for potential confounding by age and sex. Semi-dried tomatoes and fresh garlic were the only foods with statistically significant ORs (95% CIs). Neither dried or bottled garlic, nor any other food investigated, was significantly associated with illness. Eating at restaurants, however, was found to be associated with an elevated risk of illness after adjusting for sex and age (adjusted OR 3·1, 95% CI 1·2–7·8). Exposure to livestock was not significant after age and sex were adjusted for in analyses.

Twenty-two of the 32 cases had eaten semi-dried tomatoes and/or fresh garlic. Seven of those who had eaten neither of these foods did report having eaten at restaurants within the 5 days prior to illness compared with 10 of 27 controls who also reported neither garlic

Table 1. *Exposure frequencies and matched odds ratios in PT 8 cases and controls*

	Frequencies		Matched OR		Adjusted* matched OR	
Exposure	PT 8 cases	Controls	OR	95% CI	OR	95% CI
Food consumption						
Eggs	26/30	53/64	0·9	0·3–2·7	1·0	0·3–3·7
Runny eggs	9/29	13/60	1·7	0·6–4·5	2·8	0·9–9·4
Poultry	25/32	41/64	2·3	0·8–6·6	0·5	0·2–1·4
Semi-dried tomatoes	7/30	2/64	12·6	1·5–103·1	10·1	1·2–85·8
SDT ingredients†						
Fresh tomatoes	21/28	41/62	1·3	0·4–4·8	1·7	0·4–8·2
Garlic (fresh)	18/28	22/59	4·1	1·3–12·8	3·5	1·0–12·4
Parsley	7/28	12/61	1·2	0·4–3·5	1·5	0·4–5·4
Oregano	6/27	10/60	1·3	0·4–4·1	1·5	0·4–5·6
Canola oil	7/27	11/64	0·7	0·3–1·8	0·8	0·3–2·5
Raw or rare meat	3/31	12/106	0·5	0·1–1·9	0·6	0·1–2·5
Hamburger mince	11/32	24/64	0·9	0·4–2·1	0·7	0·3–1·9
Cold meats	18/31	39/64	0·9	0·4–2·5	0·9	0·3–2·6
Seafood	13/32	28/64	0·9	0·4–2·2	0·9	0·4–2·5
Garlic (bottled/dried)	7/13	10/28	1·0	0·2–4·3	1·0	0·2–5·2
Salad	12/32	20/64	1·4	0·5–7·7	1·5	0·5–4·2
Milk (unpasteurized)	1/31	1/61	2·0	0·1–32·0	1·6	0·1–26·7
Untreated water	4/31	6/63	1·5	0·3–6·7	2·2	0·4–11·5
Home-grown produce	5/29	17/63	0·7	0·2–2·0	0·4	0·1–1·5
Where food eaten						
Restaurants	17/30	18/64	3·1	1·2–7·8	3·8	1·4–10·7
Public gatherings	9/30	10/63	2·1	0·7–6·4	1·9	0·6–6·3
Take-away	15/30	28/64	1·2	0·5–2·9	1·0	0·4–2·7
Food handling						
Raw eggs	17/32	28/64	1·5	0·6–3·7	1·4	0·5–3·8
Safe food handling‡	4/32	16/64	0·4	0·1–0·4	0·3	0·1–1·5
Other exposure						
Livestock	6/31	4/64	3·6	0·9–14·5	2·8	0·7–12·1
Pets	20/31	35/64	1·6	0·6–4·4	1·7	0·6–4·8
Animal manure	8/30	12/62	1·5	0·5–4·3	1·2	0·4–3·8

* Odds ratios adjusted for sex and age using conditional logistic regression.
† Separate consumption of the foods used as ingredients of semi-dried tomatoes (SDT).
‡ Safe food handling: wash chopping board *and* knife with soap and water after using for raw meat or poultry before using for uncooked foods.

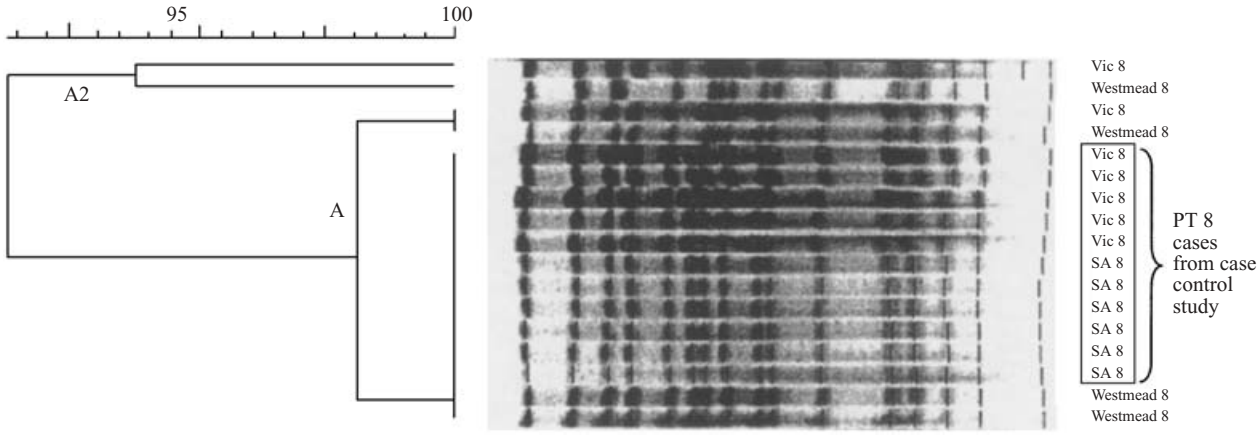

Fig. 2. Pulsed field gel electrophoresis of genomic DNA from *S.* Virchow PT 8 isolates digested with *Xba*I.

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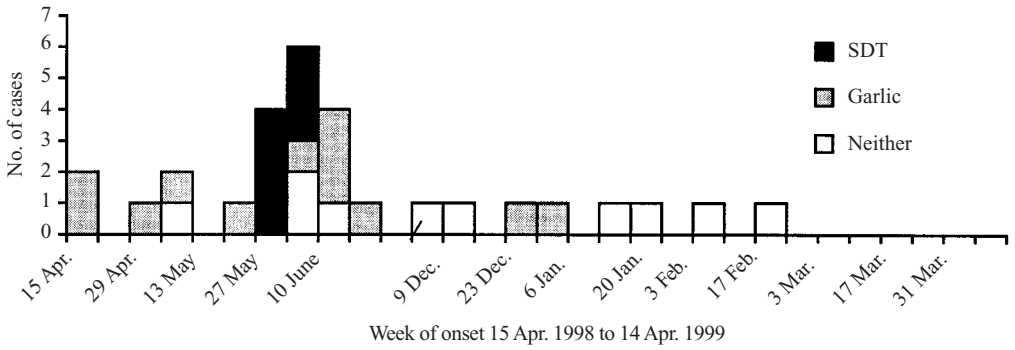

Fig. 3. Epidemic curve for PT 8 *S.* Virchow cases according to garlic and semi-dried tomato (SDT) consumption.

or semi-dried tomato exposure (OR 3·97, 95% CI 0·7–25·6). All cases that reported having consumed semi-dried tomatoes had also consumed fresh garlic. It is, therefore, not possible to examine further the epidemiological independence of these associations.

The semi-dried tomatoes consumed by cases were traced back to two different manufacturers, both located in Victoria. *S.* Virchow was isolated from opened packages of semi-dried tomatoes at two retail outlets where cases had purchased the products originating from one or other of these manufacturers. The retail outlets only carried one brand of semi-dried tomatoes each, and were located in two different states. Inspection of the production lines of the two manufacturers failed to identify evidence of mechanisms, or source(s), of contamination. However, the investigation did reveal semi-dried tomatoes to be a potential risk food as they are oven-dried at temperatures inadequate for inactivation of salmonella. In addition, potentially high-risk ingredients, such as herbs, are added to the dried tomatoes and the final product is not subjected to any further heat processing.

The association with semi-dried tomatoes was, however, transitory with the onset of illness for cases with this exposure confined to a 2 week period in late June 1998 (Fig. 3). Further investigation identified fresh, unprocessed, garlic (or fresh bulb garlic) imported from China as the only ingredient used in the two brands that was known to originate from a single distributor and source. Moreover, fresh garlic was the only ingredient of the semi-dried tomatoes (tomatoes, fresh garlic, canola oil, oregano or parsley) to also show an association with illness in our case control study. Consumption of fresh garlic explained more cases with onset dates spread over a longer time period.

Fresh garlic consumed by cases was traced back to the retail outlet where possible. Of the seven cases who reported having eaten fresh garlic other than as an ingredient of semi-dried tomatoes in the 5 days prior to illness, and for whom we had sufficient detail on retail outlets and their suppliers, six had consumed imported fresh garlic from China. It should be noted, however, that the majority of fresh garlic available for retail over the winter of 1998 was imported from China.

DISCUSSION

This investigation was motivated by the potential severity of *S.* Virchow infection, as confirmed by the fatality and the high rates of hospitalization (39%) and bacteraemia (13%). Infection resulted in more invasive illness than other salmonella serotypes notified in New South Wales at that time – isolation sites for laboratory specimens revealed that cases infected with *S.* Virchow were four times more likely to develop invasive illness than for all other salmonella infections. *S.* Virchow PT 8 was the predominant phage type associated with the increase of *S.* Virchow notifications seen in South Eastern Australia during the summer of 1997–1998.

We submitted a panel of 50 *S.* Virchow isolates to PFGE, which included strains from this and previous investigations. All human case and environmental isolates from this investigation were PFGE group A, with 95% or better similarity, and only 57% of the PT 8 strains tested were found to be PFGE group A. The inability of PFGE to differentiate among case and environmental isolates from this investigation strengthens the argument that our PT 8 cases had a common source. Further, the isolation of *S.* Virchow PT 8 from semi-dried tomatoes not only identified this intermediary vehicle but, importantly, directed us to further investigate mechanisms for contamination of this product.

The age difference between cases and controls reflects a higher incidence of salmonellosis in the younger age groups, with 28% of cases aged 12 years or less. This is the pattern expected with the increased likelihood of children with diarrhoeal illness being seen by a doctor who will then request laboratory tests, or increased susceptibility and/or severity of illness in young children. However the distribution of exposure in the community contributed to this age difference and also accounts for the gender difference between cases and controls, with young adult females more likely to consume semi-dried tomatoes.

Eating at restaurants also remained significantly associated with illness after adjusting for sex and age. Cases who ate at restaurants may not have been aware of the raw foods used in the preparation of food consumed, and so the exposure to garlic may be under-reported. This might explain the source of *S*. Virchow PT 8 for 7 of the 10 cases who reported that they had not knowingly consumed fresh garlic or semi-dried tomatoes. However, most of the 10 cases not exposed to garlic occurred in the second 6-month period of the study and so the possibility remains that they may have originated from different source(s). This also corresponded to the period when supermarket stocks of garlic were observed to switch from imported to local produce.

Livestock exposure was not a significant risk factor for infection after adjusting for sex and age but, given the limited power of this study, it remains as a possible additional local source of infection. Four of the 10 cases exposed to livestock had neither eaten semi-dried tomatoes nor fresh garlic, and two of these had also not eaten at restaurants. Exposure to garlic, restaurant food or livestock explains all but one of the PT 8 cases.

The combined findings of our epidemiological and environmental investigations identified semi-dried tomatoes as a likely vehicle for salmonella, and imported fresh garlic as a potential source for the outbreak experienced in South Eastern Australia during the summer of 1997–1998. Fresh garlic is implicated epidemiologically with elevated adjusted ORs for garlic consumption, and fresh garlic was the key common ingredient used in the two brands of semi-dried tomatoes linked to cases and from which *S*. Virchow was isolated. However, there are some things that are difficult to explain if we wish to argue that garlic caused this outbreak. First, garlic contains allicin, an antimicrobial agent. Secondly, most cases who ate fresh garlic reported eating it only after it was cooked.

Garlic (*Allium sativum*) is a perennial plant that is propagated from the cloves that form the bulb of the plant and is grown as an annual crop. It is harvested by being dug from the ground with the shoots and roots still attached, and undergoes no further processing. We were unable to isolate *S*. Virchow from the crushed fresh garlic used for the manufacture of the semi-dried tomatoes. Survival and detection of any salmonellas is exacerbated by the presence of antimicrobial factors. Garlic, through the action of the compound diallyl thiosulphinate (allicin), possesses antimicrobial properties [14] and the routine salmonella detection requires prior neutralization of these inhibitory substances [15].

We now know that salmonella can survive on the external surfaces of garlic from a recent study of the effects on crops of irrigation with treated wastewater [16]. Garlic was found to reach sanitary acceptability 90 days after harvest, once attached roots and soil were removed. The imported garlic available during the period of this study came from a region where crops may be fertilized with untreated human waste and, whilst the garlic appeared clean, the roots were still attached.

However surface contamination alone may not pose a significant risk if the garlic is cooked. It is possible that *S*. Virchow infection resulted from consumption of foods other than garlic eaten raw after being contaminated by fresh garlic in storage or during food handling. The potential for cross-contamination as a mechanism for food contamination from fresh garlic warrants further attention, and requires more detailed information on the way garlic is prepared and consumed. It would also be useful to broaden food-handling questions to investigate the potential for cross-contamination during food preparation from foods other than raw meat (Table 1, third footnote).

We have more convincing epidemiological, environmental and microbiological evidence to implicate semi-dried tomatoes as a vehicle. The limited consumption of this food in the general population allowed us to recognize the potential for this to be a vehicle when only a few of the cases had been interviewed and were found to have this exposure. It also assisted in the quality of recall and therefore minimized misclassification. Most consumers either clearly linked semi-dried tomato exposure to a special occasion, or were very regular consumers. Non-consumers were usually very certain that they had not eaten this product within the period under question.

S. Virchow PT 8 was successfully isolated from different brands of semi-dried tomatoes at two separate retail outlets linked to two cases. Whilst we cannot rule out contamination after opening, the likelihood of this occurring with a rare serotype in two independent incidents during one outbreak is extremely remote.

In summary, the evidence for semi-dried tomatoes is:

(1) epidemiologically, there was a strong association with semi-dried tomatoes;
(2) microbiologically, *S*. Virchow PT 8 was isolated from two opened containers of semi-dried tomatoes at retail outlets in separate states;
(3) on environmental traceback, the two containers of opened semi-dried tomatoes were found to be from different manufacturers, but did share a single common ingredient – fresh bulb garlic imported from China.

However, we do not have sufficient evidence to prove that fresh garlic was the original *S*. Virchow PT 8 source in the semi-dried tomato manufacturing process. Fresh tomatoes themselves have been associated with salmonella outbreaks previously [17], but the tomatoes used by the manufacturers of the semi-dried tomato products linked to cases in our investigation were from different distributors and originated from different local growers. Fresh garlic is the only ingredient of the semi-dried tomatoes that was from a common source and for which we do not have to hypothesize that there was more than one contamination event from a rare serovar within Australia. We cannot prove that garlic was the vehicle in this *S*. Virchow PT 8 outbreak, but sufficient evidence and plausible mechanisms have been identified here to warrant further investigation of fresh garlic and semi-dried tomatoes as potential vehicles for *Salmonellae*.

We recommend that fresh garlic and non-heat-treated foods such as semi-dried tomatoes be considered as possible vehicles in future salmonella outbreak investigations. As the fresh garlic under investigation here was imported, there are global implications for both producers and consumers of fresh garlic and other fresh produce, and we encourage international collaboration to describe the origins and global distribution of fresh garlic.

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