

## An outbreak of *Salmonella* Typhimurium 9 at a school camp linked to contamination of rainwater tanks

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### SUMMARY

In March 2007, an outbreak of gastroenteritis was identified at a school camp in rural Victoria, Australia, affecting about half of a group of 55 students. A comprehensive investigation was initiated to identify the source. Twenty-seven attendees were found to have abdominal pain, diarrhoea and nausea (attack rate 49%). Of 11 faecal specimens tested all were positive for *Salmonella* Typhimurium definitive phage type 9 (DT9). Of four samples taken from the untreated private water supply, two were positive for DT9. Drinking water from containers filled from rainwater tanks [relative risk (RR) 3·2,  $P=0\cdot039$ ] and participation in two recreational activities – flying fox (RR 5·3,  $P=0\cdot011$ ), and beam-balance (RR 3·9,  $P=0\cdot050$ ) – were indicative of a link with illness. Environmental and epidemiological investigations suggested rainwater collection tanks contaminated with DT9 as being the cause of the outbreak. Increased use of rainwater tanks may heighten the risk of waterborne disease outbreaks unless appropriate preventative measures are undertaken.

**Key words:** Australia, disease outbreak, salmonellosis, water.

### INTRODUCTION

In March 2007, the Victorian Government Department of Human Services became aware of an outbreak of gastroenteritis which had occurred at a school camp in rural southeast Victoria. The school Principal reported that about half attendees had

become ill with diarrhoea and vomiting. *Salmonella* Typhimurium definitive phage type 9 (DT9) was isolated from faecal samples of all 11 students tested.

In Victoria, DT9 has been one of the three most common phage types affecting humans, and has dominated the state's *Salmonella* profile for more than 30 years [1, 2]. Like other *S.* Typhimurium phage types, DT9 was previously associated with a variety of sources including environmental, domestic and native animals and live poultry [3]; and was also identified in a variety of food sources, including red meat, salami, chicken, and eggs [4].

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In this report, we describe an unusual outbreak of DT9 that occurred as a result of drinking contaminated water from a private water supply.

## METHODS

### Setting

The camp facility in rural Victoria was situated on 80 acres of bushland about a 10-min drive from the nearest town centre. The facility was used for various purposes, including school camps, corporate retreats, and other private group functions. Many of the numerous buildings on the site were in a state of disrepair. The camp was operated by an extended family and a small group of employees, most of whom lived in the various buildings situated on the property. School camp groups were restricted to the communal building and the six accommodation cabins. Many of the recreational activities were conducted in and around a large lake. The communal building was in the centre of the property, and included a large dining room (seating capacity for up to 100 people) and adjoining recreation room, industrial-sized kitchen, public toilets, and a laundry. A small staff quarters was attached to the kitchen.

### Case definition

A case was defined as a student, teacher or parent of the school group who attended the camp between Tuesday 27 February and Friday 2 March 2007 and who had acute gastroenteritis (nausea and/or abdominal pain and/or diarrhoea) with onset between Wednesday 28 February and Monday 12 March.

### Environmental investigation

A comprehensive investigation of the camp facility included an assessment of food preparation techniques, interviews with staff, review of food safety standards, and sampling of the private water supply.

A map of the property was obtained from the camp proprietors showing the location of camp buildings and accommodation cabins, staff quarters, lakes, dams, and recreational activities. The design and operation of the private water supply was described by the camp proprietor. Water samples were obtained from four sites on the property, although this was about 2 weeks after the start of the outbreak. They included one each from the kitchen and bathroom taps in the communal building, and

two from one tap and one of the rainwater collection tanks attached to one of the cabins. No leftover food was available for sampling.

### Laboratory investigation

Faecal specimens were tested at the Microbiological Diagnostic Unit (the national phage-typing reference laboratory) based at the University of Melbourne. They were analysed for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Aeromonas*, and *Plesiomonas* spp. by routine bacteriological methods. Stool samples were cultured for *Salmonella* using selective media, and confirmed as DT9 using the Institut Pasteur serotyping designation and the Colindale *Salmonella* Typhimurium Phage Typing Scheme [5, 6]. Antibiotic susceptibility testing [7] and MLVA typing were also conducted. MLVA was performed as described by Lindstedt *et al.* [8] with the following modifications. Five colonies of *S. Typhimurium* were resuspended in 100  $\mu$ l TE and boiled for 10 min. The supernatant was used in a modified multiplex reaction using Qiagen multiplex mix (Qiagen, Hilden, Germany). M1 consisted of 0.4  $\mu$ M STTR3 and 0.05  $\mu$ M STTR6 primers, and M2 consisted of 0.1  $\mu$ M STTR5, STTR10pl and 0.05  $\mu$ M STTR9 primers. The reactions were run on an ABI-3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) using POP-4 (Applied Biosystems), and the Geneflo-625 ladder (ChimerX, Milwaukee, WI, USA). The results were analysed using GeneScan and Genotyper (Applied Biosystems). Allele numbers were assigned according to a previously used scheme [8], and reported in the order STTR9–STTR5–STTR6–STTR10pl–STTR3.

### Epidemiological investigation

#### Study design

A retrospective cohort study was conducted to identify any association between illness and specific exposures at the camp. All staff, students, and parents who attended the camp from Tuesday 27 February to Friday 2 March were included in the study. A questionnaire was designed based on the information obtained from the camp proprietors, and included 109 exposure-related variables (84 food items, 10 water and 15 recreational activities). Detailed information was also collected on any water consumed, including whether it was obtained from onsite storage containers or from personal water bottles (and how they were filled). Questionnaires were self-administered,

with assistance from the Environmental Health Officer involved in the investigation.

### Data analyses

Questionnaire data were entered into a Microsoft Access database and then analysed using Stata version 9.0 (StataCorp., College Station, TX, USA). Univariate analyses were conducted, and Fisher's exact test, with  $P < 0.05$  was considered indicative of a statistically significant finding.

## RESULTS

### Environmental investigation

Three rainwater collection tanks supplied water to the main camp communal building. The catchment area for all three tanks was the roof of the communal building.

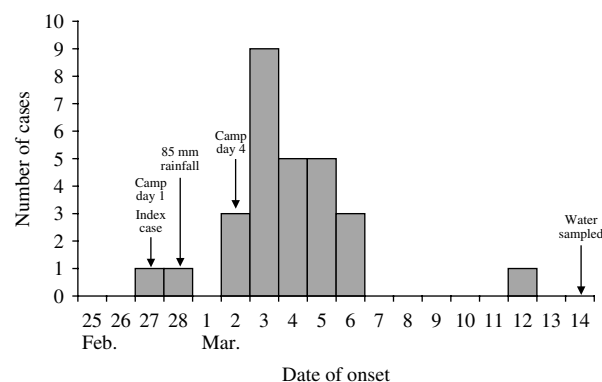
One tank was attached to the laundry and bathroom. Two tanks (situated in dense bush at the side of the building) supplied the kitchen and were used for drinking water. Rainwater for drinking from the communal building tanks was transported in 15-litre containers to the cabins.

A large lake was situated about 1 km to the east of the communal building. There were six accommodation cabins between this lake and the communal building. Rainwater tanks were attached to each of these accommodation cabins and used to supply water for showering and handwashing. A water pump situated on the shore of the large lake had previously been used to pump lake water to 'top-up' these tanks, as in the 12 months prior to the outbreak there had been very little rain. This practice had, however, not been necessary for many months prior to the outbreak.

No chlorination of any water source was undertaken at the camp facility.

### Laboratory investigation

*Salmonella* was cultured from all 11 stool samples submitted for examination from patients with gastroenteritis. No other pathogens were isolated. In addition, *Salmonella* was recovered from two of the four tap-water samples collected, both of which were from the communal camp building (kitchen tap and the female-bathroom tap). All positive samples were serotyped as *S.* Typhimurium and phage-typed as DT9. All isolates were found to be sensitive to all



**Fig. 1.** Cases of gastroenteritis, by date of illness onset, 27 February to 12 March 2007, southeast Victoria ( $n = 27$ ).

antibiotics tested. MLVA typing found that all 13 of the positive isolates displayed an identical and infrequently seen pattern, supporting a link between these cases.

### Epidemiological investigation

Forty-five students and ten adults (nine teachers and one parent) attended the camp facility between Tuesday 27 February and Friday 2 March and were included in the cohort study. Of the students, 21 were female and 24 were male (age range 12–15 years, median age 13.3 years). Of the adults, five were female (age range 38–57 years, median age 47.3 years).

Twenty-seven of the 55 camp participants met the case definition for illness (attack rate 49%). Although not included in the analytical epidemiological study, it was subsequently ascertained that the first case in the outbreak was a young child of a family living at the camp facility, who became ill on 27 February, the first day of the camp. A faecal sample from the child was positive for DT9.

In the school group, most cases had onset between Friday 2 March and Tuesday 6 March, except for one student who became ill on Monday 12 March, 10 days after the last day of the camp (Fig. 1). This outlier case was not laboratory confirmed, and although her illness was fairly short-lived (3 days), her reported symptoms met the case definition for illness. The reason for this outlier is not clear, but secondary transmission from one of the other camp attendees could not be ruled out. The first case in the school camp group became ill on 28 February (day 2 of the camp), with the greatest number of cases ( $n = 9$ ) becoming ill on 3 March. The epidemic curve was suggestive of a point-source exposure causing this outbreak.

Table 1. Univariate analysis of exposures among 27 cases and 28 controls, southeast Victoria, March 2007

| Item                            | Exposed   |       |        | Not exposed |       |        | RR  | (95% CI)   | P value* |
|---------------------------------|-----------|-------|--------|-------------|-------|--------|-----|------------|----------|
|                                 | No. cases | Total | AR (%) | No. cases   | Total | AR (%) |     |            |          |
| Dinner, day 1                   |           |       |        |             |       |        |     |            |          |
| Roast potato                    | 24        | 44    | 54     | 2           | 8     | 25     | 2.1 | (0.6–7.5)  | 0.248    |
| Bread                           | 23        | 40    | 57     | 3           | 12    | 25     | 2.3 | (0.8–6.3)  | 0.097    |
| Oranges                         | 4         | 13    | 30     | 22          | 37    | 59     | 0.5 | (0.2–1.2)  | 0.109    |
| Breakfast, day 2                |           |       |        |             |       |        |     |            |          |
| Day 2 breakfast (all foods)     | 25        | 42    | 59     | 2           | 9     | 22     | 2.7 | (0.8–9.3)  | 0.066    |
| Peanut butter                   | 1         | 5     | 20     | 24          | 43    | 45     | 0.4 | (0.1–2.1)  | 0.180    |
| Lunch, day 2                    |           |       |        |             |       |        |     |            |          |
| Hommus                          | 1         | 6     | 16     | 24          | 44    | 54     | 0.3 | (0.1–1.9)  | 0.189    |
| Dinner, day 2                   |           |       |        |             |       |        |     |            |          |
| Apple sponge                    | 9         | 27    | 33     | 16          | 23    | 69     | 0.5 | (0.3–0.9)  | 0.022    |
| Lunch, day 3                    |           |       |        |             |       |        |     |            |          |
| Tossed salad                    | 8         | 25    | 32     | 18          | 25    | 72     | 0.4 | (0.2–0.8)  | 0.010    |
| Dinner, day 3                   |           |       |        |             |       |        |     |            |          |
| Day 3 dinner (all foods)        | 24        | 41    | 58     | 2           | 9     | 22     | 2.6 | (0.7–9.2)  | 0.069    |
| Tossed salad                    | 9         | 27    | 33     | 17          | 25    | 68     | 0.5 | (0.3–0.9)  | 0.025    |
| Breakfast, day 4                |           |       |        |             |       |        |     |            |          |
| Canned spaghetti                | 13        | 18    | 72     | 13          | 31    | 41     | 1.7 | (1.0–2.8)  | 0.074    |
| All drinks                      |           |       |        |             |       |        |     |            |          |
| Orange cordial                  | 25        | 44    | 56     | 2           | 9     | 22     | 2.5 | (0.7–8.9)  | 0.076    |
| Water from containers in cabins | 25        | 43    | 58     | 2           | 11    | 18     | 3.2 | (0.9–11.5) | 0.039    |
| Activities                      |           |       |        |             |       |        |     |            |          |
| Snowy Challenge                 | 24        | 40    | 60     | 3           | 11    | 27     | 2.2 | (0.8–5.9)  | 0.088    |
| Touch Pad                       | 26        | 46    | 56     | 1           | 7     | 14     | 3.9 | (0.6–24.7) | 0.050    |
| Rock Wall                       | 26        | 46    | 56     | 2           | 9     | 22     | 2.5 | (0.7–8.9)  | 0.076    |
| Tatiana                         | 25        | 44    | 56     | 2           | 9     | 22     | 2.5 | (0.7–8.9)  | 0.076    |
| Zip                             | 26        | 44    | 59     | 1           | 9     | 11     | 5.3 | (0.8–34.3) | 0.011    |

AR, Attack rate; RR, relative risk; CI, confidence interval.

\* The *P* value reported has been calculated using Fisher's exact two-tailed test of significance.

The most common symptom experienced by cases in the outbreak was abdominal pain ( $n=22$ , 82%), followed by diarrhoea ( $n=19$ , 70%) and nausea ( $n=16$ , 59%). Other symptoms reported were headache ( $n=19$ , 70%), fever ( $n=14$ , 52%), anorexia ( $n=13$ , 48%), and lethargy ( $n=13$ , 48%). Cases were ill for an average of 4.8 days (range 1–12 days). Three of the cases attended a hospital emergency department as a result of their illness, but none was admitted. A further seven cases indicated that they had consulted a general practitioner.

Univariate analyses of the association between exposures and illness (Table 1) indicated that there were five food items with a relative risk >2.0 (roast

potato, bread, breakfast on day 2, dinner on day 3, and canned spaghetti on day 4), but none of these were statistically significant. A significant association, however, was found between illness and drinking water from storage containers in the cabins [relative risk (RR) 3.2, 95% confidence interval (CI) 0.9–11.5,  $P=0.039$ ]. The 15-litre semi-transparent containers were clearly marked 'drinking water only', and kept on the kitchen sinks in the cabins. The containers were refilled on a daily basis from the tap in the communal building kitchen (supplied by rainwater tanks) and transported back to the cabins for use.

Analyses also suggested an association between illness and participation in two recreational activities.

A high-wire swing activity suspended across the large lake on the property (Zip) gave a high relative risk which was statistically significant (RR 5.3, 95% CI 0.8–34.3,  $P=0.011$ ). A beam-balance exercise (Touch Pad) also gave a high relative risk, but was only found to be of borderline statistical significance (RR 3.9, 95% CI 0.6–24.7,  $P=0.050$ ).

## DISCUSSION

In this paper we describe an outbreak of DT9 at a school camp which appears to have been caused by contamination of rainwater tanks supplying drinking water. Although infection with DT9 is common both in Victoria and Australia, and has previously been linked to various sources, to our knowledge this is the first time this organism has been associated with water consumption. Our investigation highlights the potential increased disease risk that may result from the more frequent use of private water tanks in Australia.

We hypothesized that the cause of this outbreak was contaminated water sourced from large rainwater tanks which supplied drinking water to the camp. This was supported by the isolation of DT9 from 11 human cases, as well as from two water samples from taps supplying drinking water. Moreover, the epidemiological study was suggestive of an association between consumption of water (from storage containers filled from the rainwater tanks) and illness. We were able to obtain supportive environmental evidence despite the fact that water samples were taken 2 weeks after the start of the outbreak, and despite the obvious difficulties in demonstrating the association between a ubiquitous exposure such as water consumed from a single source, and illness. The identification of the pathogen from both drinking water and clinical samples, along with supportive epidemiological evidence, satisfies the criteria established by Tillet *et al.* [9] that the outbreak was ‘strongly associated’ with water from the rainwater tank. A similar outbreak of salmonellosis at a school camp was reported in 1978 in Trinidad [10]. In this outbreak, it was postulated that rainwater which collected on the surface of the kitchen/dining hall roof and was stored in a single tank, was contaminated by bird faeces containing *Salmonella arechevalata*.

Several additional findings from the environmental investigation support the hypothesis of drinking water as the source of this outbreak. First, contrary to legislation governing such facilities, the camp

management had not chlorinated the water supply, nor had they regularly ‘desludged’ or disinfected the rainwater tanks on the property. The reasons for this, at least in part, appear to have been due to the strict religious beliefs of the camp owners, which prohibited the addition of chemicals to water. Second, the observed state of the rainwater tanks supplying drinking water indicated that, although the tank openings were protected with gauze, the tanks were situated in dense scrub bush, predisposing them to contamination from dirt, leaf debris and animal droppings. Third, at the time of the outbreak, the region where the camp facility was situated was in the midst of a severe drought, with areas around the camp facility registering some of the lowest rainfall levels on record. However, on the first day of the camp, 85 mm of rain was recorded in the region during a 24-h period [11], washing accumulated debris from the roof of the main building into the rainwater tanks. It is widely reported that the greater the interval between rain events, the poorer the quality of water washing into rainwater tanks [12–16].

A statistically significant association was found between participation in one of the recreational activities undertaken at the camp and illness, this being a flying-fox activity across the large lake (Zip). Although there was no obvious link between this activity and the consumption of water, containers of water (sourced from the contaminated supply) were made available to the group participating in this activity. One possibility is that those who participated in the activity consumed more water than others, thus increasing their likelihood of illness, although this does not explain why other physical activities undertaken at the camp were not also associated with illness.

Although many *Salmonella* serovars can survive in water, information from Australia’s national register of enteric pathogens suggests that DT9 would only be found in water if it had been the subject of a recent contamination, an occurrence which has only once been reported previously in Australia [1–3]. In this instance, DT9 was isolated as part of routine monitoring by the local water company from a stream in Victoria which was receiving agricultural runoff (beef and dairy cattle are the main domestic stock farmed in the area). No source was identified at the time, although DT9 had also been isolated from dairy cows in the same region at around the same time. Water contamination events with other *Salmonella* serovars however have been reported both

in Australia [17–19], and internationally [10, 20], albeit rarely. All of these have been associated with contamination by infected wildlife [10, 19], close proximity to animal populations [16, 17, 20], or soil or plant material [18].

In conclusion, this paper describes an outbreak of gastroenteritis caused by contamination of a private drinking water supply with DT9. We consider that a number of factors contributed to this outbreak, including the occurrence of a severe and extended drought followed by a major rain event, the accessibility of rainwater tanks to animal matter, and the failure to treat water by chlorination or other means. With the severity of drought seemingly on the rise in Australia, and the ensuing focus on water conservation, particularly on basic water-saving measures, more Australians than ever before are installing rainwater collection tanks [21]. Consequently there is a need for the potential health risks of these storage tanks to be better understood and documented, and that this is used to inform the implementation of appropriate regulatory measures.

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## DECLARATION OF INTEREST

None.

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