AN INSTITUTIONAL OUTBREAK OF *SALMONELLA ENTERITIDIS* IN SINGAPORE

DPK Ng[1], KT Goh[1], MGC Yeo[2] and CL Poh[3]

[1]Quarantine and Epidemiology Department, Ministry of the Environment, Singapore; [2]Department of Pathology, Singapore General Hospital; [3]Department of Microbiology, National University of Singapore

Abstract. A large outbreak of food poisoning occurred in Singapore in March 1995 when a total of 188 inmates in an institution was taken ill. *Salmonella enteritidis* was isolated from the stool cultures of 35 inmates (16 symptomatic and 19 asymptomatic). All the isolates were of the serotype profile 0:1, 9, 12 and H:g, m (antigen phase I); all were sensitive to ampicillin, ceftriaxone, chloramphenicol, co-trimoxazole and ciprofloxacin. Plasmid profile analysis and restriction enzyme fragmentation patterns (REFPs), as generated with EcoRI and HindIII, of a 60 kb plasmid obtained from these isolates were all identical, confirming that the outbreak resulted from a single source of infection. Stratified statistical analysis of food-specific attack rates strongly implicated imported canned luncheon pork consumed by the inmates on 26 March 95 as the single most probable cause of the food poisoning [$p < 10^6$, Mantel-Haenszel weighted odds ratio (OR) = 14.33; 95% confidence interval (CI) = 6.20 - 33.15]. The median incubation period of this outbreak was 19.3 hours and the median duration of illness was three days. The outbreak was rapidly brought under control through prompt implementation of epidemic control measures which comprised active search for diarrheal cases, rectal swabbing of asymptomatic inmates, isolation of those found to be infected, and maintenance of a high standard of personal, food and environmental hygiene.

INTRODUCTION

Salmonella enteritidis has established itself as a major cause of foodborne illness worldwide with many countries witnessing substantial increases in its isolation rate over the past decade (Rampling, 1993). In the United Kingdom, a large increase in *S. enteritidis* (mainly phage type 4) infection was observed beginning 1985 (Cooke, 1991). Isolations in England and Wales had more than tripled from 2,071 in 1981 to 6,858 in 1987 (Coyle *et al*, 1988). The increasing incidence was largely attributed to consumption of raw or undercooked contaminated poultry and hen eggs (Bartlett *et al*, 1989). Similarly, in the United States, a five-fold increase in the isolation of *S. enteritidis* was noted in New England and the Middle Atlantic region between 1976 and 1985. By 1985, *S. enteritidis* had displaced *S. typhimurium* as the most frequently isolated serotype in northeastern United States. The main vehicles of transmission were poultry, eggs and egg-containing products with 65 of the 140 outbreaks that occurred there between 1985 and 1988 being attributed to grade A shell eggs (Centers for Disease Control, 1987). Strains of *S. enteritidis* phage type 4 have also been isolated from cattle and pigs as well as human food not of poultry origin (Threlfall *et al*, 1994).

In Asian countries such as Japan (Ministry of Health and Welfare, 1995), Hong Kong (Wong *et al*, 1994), Malaysia (Yasin *et al*, 1995) and Thailand (Kantama and Jayanetra, 1996), the frequency of isolation of *S. enteritidis* has also increased during the period 1989-1991. In Singapore, *S. typhimurium* continued to be the prominent non-typhoidal salmonella serotype isolated from 1972 to 1993 until it was replaced by *S. enteritidis* in 1994 and 1995. The latter now accounted for between 32.8% and 36.9% of all non-typhoidal *Salmonella* isolates (Dept of Pathology, 1994). Although the enteropathogen has also been isolated from animal sources such as chicken and duck meat, hen eggs and pork sausages (Australian Salmonella Reference Centre, 1995), these food items have not been implicated in human salmonellosis caused by *S. enteritidis* in Singapore as the reported cases occurred singly and sporadically.

The isolation of *S. enteritidis* in a large outbreak of food poisoning which occurred in an institution in March 1995, provided an opportunity to determine the vehicle of transmission of infection.

Correspondence: Dr Goh Kee Tai, Head, Quarantine and Epidemiology Department, Ministry of the Environment, #22-00, 40 Scotts Road, Singapore 228231.

The institution

This was a penal institution situated in a suburban area. It had an all-male population of 1,800 housed in six blocks of dormitories served with modern sanitation. A hospital located within the premises provided outpatient and in-patient treatment for the inmates.

Three meals were prepared daily by 56 cooks and their assistants in three in-house kitchens. The kitchens were well equipped with modern sanitary and refrigeration facilities. Food items were cooked in batches and placed in individual plastic food trays before being delivered by trolleys to the various dormitories. Food was then consumed in a dining room within each dormitory. For security reasons, food was eaten using bare hands.

The outbreak

On 29 March 95, the Department of Pathology, Singapore General Hospital, noted a sudden increase in the isolation of *S. enteritidis* from the stool cultures submitted by the institution. It was isolated from 7 inmates who presented with symptoms of food poisoning on 26 March 1995. When notification was received by the Quarantine and Epidemiology Department, Ministry of the Environment, epidemiological investigation was immediately conducted to determine the extent of the problem and the vehicle of transmission. Control measures were concurrently taken to prevent further transmission of infection.

Preliminary investigations showed that sporadic cases of diarrhea were reported by the hospital located within the institution until 26 March 1995 when 12 inmates were taken ill. Cases were confined to inmates housed in three of the dormitories and served with food prepared in one of the kitchens. None of the security and hospital personnel who obtained their food from the staff canteen were affected.

Active search for other unreported cases was carried out and those with gastrointestinal symptoms were isolated and treated. The environmental sanitation in the institution was stepped up and food handlers instructed to observe strict personal and food hygiene. Food and water samples were collected for microbiological analyses. Rectal swabs were also obtained from asymptomatic inmates of the affected dormitories, as well as from all the 56 cooks and kitchen assistants, and submitted to the laboratory for isolation of enteropathogens.

MATERIALS AND METHODS

A structured questionnaire survey was administered to all the inmates staying in the three affected dormitories to elicit specific clinical symptoms and details of food history during the three-day period between 24 March and 26 March 1995. A case of food poisoning was defined as an individual who had consumed food provided by the institution and subsequently developed diarrhea and other gastrointestinal illness during the period 24 March to 29 March 1995. Those with no diarrhea were not included in the statistical analyses.

For statistical analysis of the food-specific attack rates, differences in proportions were compared by chi-square test using Yates' continuity correction. Fisher's exact test was employed in cases where the chi-square test was not appropriate. Stratified analysis was by Mantel-Haenszel chi-square test. Probability (p) values less than 0.05 were considered statistically significant.

No food remnants from meals consumed over the period 24 March to 26 March 1995 were available. Nonetheless, several similar food items such as shell eggs, uncooked mutton, chicken, vegetable, pork and fish were collected and analysed for the presence of *Salmonella* organisms (Food and Drug Administration Bacteriological Analytical Manual, 1992). An unopened can of luncheon pork, an imported product from China, of the same batch number as that consumed by the inmates for lunch on 26 March 1995, was also tested for enteropathogens.

Stools were inoculated onto blood agar plates, two types of selective agar plates, namely, MacConkey and Salmonella-Shigella, as well as Selenite F, an enrichment broth for *Salmonella* spp. Rectal swabs were inoculated directly into Selenite F broth. The inoculated plates and tubes of broth were incubated overnight at 35°C. The inoculation plates were read the next day and four to five suspicious non-lactose-fermenting colonies from the selective plates were picked for biochemical investigations. A single colony was inoculated into Kligler iron agar and lysine-indole motility media. The tubes of

biochemical media were incubated overnight at 35°C. A loopful of the overnight incubated Selenite F broth from each tube was inoculated onto MacConkey agar and Salmonella-Shegella agar. The plates were incubated overnight at 35°C and the following day, suspicious non-lactose-fermenting colonies were picked for biochemical investigations as described above.

When the biochemical reactions indicated the presence of *Salmonella* spp, it was confirmed by slide agglutination. A suspension of the isolate from the Kligler iron agar was mixed with a loopful of polyvalent O antiserum on a glass slide. If agglutination occurred, the process was repeated with the polyvalent H antiserum. Positive agglutination confirmed that the culture was *Salmonella* spp. The culture was then serotyped by the above agglutination method with the various antisera for *Salmonella* (American Society of Microbiology, 1994). Antimicrobial susceptibility testing was then carried out on *Salmonella* spp isolates by the disk diffusion method (National Committee for Clinical Laboratory Standards Documents, 1993). The antibiotics employed were ampicillin, ceftriaxone, chloramphenicol, co-trimoxazole and ciprofloxacin.

Plasmid DNA was obtained using the modified method of Birnboim and Doly, (1979). Agarose gel electrophoresis (AGE) of the extracted plasmid DNA was carried out in 0.7% agarose in TAE solution (40 mM Tris-acetate, pH 7.9) at 80 V for two hours, with RP4 as a reference plasmid of molecular size 60 kb (Lanka *et al*, 1983). After AGE, the agarose gel was uniformly stained in ethidium bromide solution (0.1 mg/ml) and then visualised on an ultraviolet light trans-illuminator. The stained gel was photographed using a Polaroid MP4 camera with a red filter and type 55 Kodak film. Further discrimination of the plasmid DNA was achieved by complete digestion with restriction endonucleases EcoRI and HindIII (Boehringer-Mannheim, Mannheim, Germany) which were used in accordance to the manufacturer's instructions, followed by AGE to yield restriction enzyme fragmentation patterns (REFPs).

RESULTS

Of 764 inmates given the questionnaire, 635 (83.1%) responded. Of these, 188 met the case definition of food poisoning, giving an overall attack rate of 29.6%. The clinical symptoms were diarrhea (100%), abdominal cramps (90.4%), fever (78.7%), vomiting (26.6%) and nausea (48.4%). The median duration of illness was three days.

S. enteritidis was isolated from the stool cultures of 12 symptomatic inmates. It was also isolated from 23 (3.8%) of 600 rectal swabs collected from the inmates, 4 symptomatic and 19 asymptomatic. There was thus a total of 35 bacteriologically confirmed cases (16 symptomatic and 19 asymptomatic) in this outbreak. All the food handlers, were free from *Salmonella* infection. No enteropathogens were isolated from all the food and water samples.

All the 35 *S. enteritidis* isolates were of the serotype profile 0:1, 9, 12 and H:g, m (antigen phase I) and sensitive to ampicillin, ceftriaxone, chloramphenicol, co-trimoxazole and ciprofloxacin. Plasmid profile analysis revealed the presence of a 60 kb plasmid in each of the 35 *S. enteritidis* isolates with REFPs of this plasmid, as generated with EcoRI and HindIII, proving to be indentical following AGE. This plasmid probably corresponds to the serotype-specific virulence plasmid (Platt *et al*, 1988).

Analysis of the food-specific attack rates based on all the 96 different food items served during the period 24 March to 26 March 1995 implicated lean pork cubes served for dinner on 24 March and ground luncheon pork served for lunch on 26 March. Cases were more likely to report having consumed lean pork cubes than non-cases (OR 10.95; 95% CI 6.35 - 19.10). Cases were also more likely to report having consumed luncheon pork than non-cases (OR 22.45; 95% CI 10.83 - 48.15). Stratified analyses showed that cases were still more likely to report consuming luncheon pork than non-cases after controlling for lean pork cubes consumption (p - value $< 10^6$; Mantel-Haenszel weighted OR = 14.33; 95% CI = 6.20 - 33.15). However, cases were not statistically significantly more likely to report eating lean pork cubes after controlling for luncheon meat consumption (p = 0.8; Mantel-Haenszel weighted OR = 0.83; 95% CI = 0.13 - 5.13).

The lunch served on 26 March 1995 consisted of steamed rice, luncheon pork, corned mutton and brinjal. About 50 cans of luncheon pork were used

for the preparation of this food item. The contents were emptied into a container, sliced into small pieces and then left uncovered at room temperature for at least 4 hours at ambient temperature before it was warmed up and served.

The median incubation period based on the lunch served on 26 March 1995 at 12.00 noon was 19.3 hours, and ranged from one hour to 77 hours. The majority of the reported cases (73.9%) became ill 12 hours after consumption of the implicated food (Fig 1).

Environmental investigations showed that the kitchen where the implicated food item was prepared was not well maintained, providing favorable conditions for cross-contamination between raw and cooked food. Food was allowed to be stored at ambient temperature for prolonged period. All the food handlers had not attended any basic food hygiene course. None of them admitted to any recent episode of diarrhea.

DISCUSSION

The incidence of foodborne diseases in Singapore is low, mainly due to the high standards of environmental and food hygiene (Yew *et al*, 1993). However, outbreaks of food poisoning, shigellosis, typhoid and cholera had occurred in institutions where there were lapses in the personal and food hygiene of the food handlers in the kitchen (Goh *et al*, 1987), or where the personal hygiene of the inmates was poor (Goh *et al*, 1990; 1992). Outbreaks of foodborne disease in institutions are usually explosive, as in this outbreak.

Molecular epidemiological data confirmed that this outbreak of *Salmonella* food poisoning was due to infection from a single source, as evidenced by the identical REFPs of the isolated 60 Kb plasmid extracted from all the 35 *S. enteritidis* isolates. Epidemiological investigations pointed strongly to an association between illness and consumption of luncheon pork.

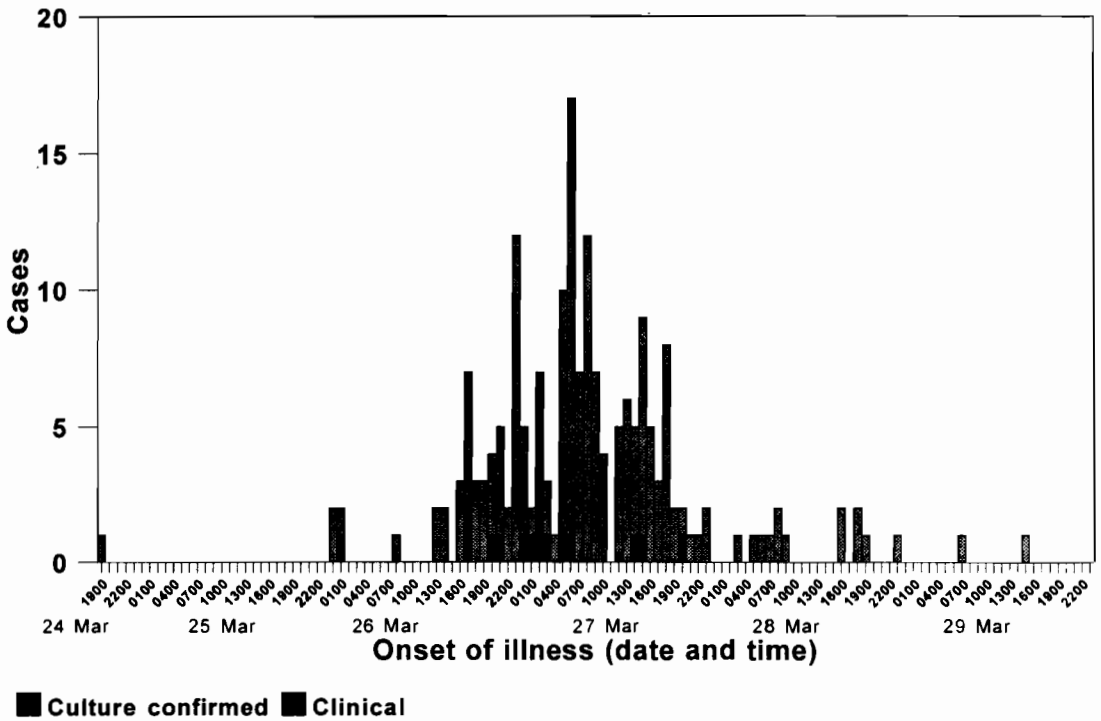

Fig 1–Date of onset of diarrhea of 188 cases of *S. enteritidis* food poisoning in an institutional outbreak in Singapore, March 1995.

The exact mechanism by which the implicated food was contaminated could not be determined. We have no evidence that the imported canned product was contaminated at the source of production, as there were no other reported cases of salmonellosis associated with the consumption of this food item elsewhere, and no enteropathogen was detected in an unopened can with the same batch number as those served for lunch on 26 March 1995. No *Salmonella* bacteria were detected in all the other food samples tested, including shell eggs.

Our observations on the personal and food hygiene practices at the institution's kitchen suggested that the implicated food was most probably contaminated during preparation and storage. The preparation of luncheon pork involved considerable handling. No specific food handler was assigned to slice the ground pork after it was emptied from the can. As all the food handlers had not attended any food hygiene course, they were not aware that special precautionary measures should be taken to prevent cross-contamination, which could occur through the fingers, kitchen utensils and work surfaces (Humphrey *et al*, 1994). As the infective dose of *Salmonella* bacteria is lower in high-fat food (Blaser and Newman, 1982), such as luncheon pork, minimal contamination of the food followed by subsequent storage at ambient temperature could result in epidemic transmission. Subsequent warming to temperatures below 60°C prior to consumption would not be adequate to inactivate all the organisms.

This outbreak would not have been recognised were it not for the vigilance of the microbiology laboratory. Subsequent epidemiological investigations and prompt implementation of epidemic control measures comprising surveillance of diarrheal cases, rectal swabbing of asymptomatic inmates, isolation of those found to be infected, and strict enforcement of a high standard of personal, food and environmental hygiene among the food handlers and inmates prevented further transmission of infection in the institution.

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