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# Detection of a nosocomial outbreak of salmonellosis may be delayed by application of a protocol for rejection of stool cultures

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**Summary** In October 2001 an outbreak of *Salmonella enterica* serovar *enteritidis* phage-type 6 occurred in a hospital and a nursing home, both served by the same hospital kitchen. Five nursing home residents died during the outbreak. *S. enteritidis* was isolated from three of them. Of 231 stool samples from nursing home residents, hospital patients and employees, 82 were culture-positive. All symptomatic patients were treated with oral ciprofloxacin. Inspection of the kitchen showed that during preparation of the desserts implicated in causing the outbreak, temperatures were not measured and storage temperatures were too high. No left-over food samples were available for analysis. According to the 'four-day rule' in use in this hospital, the stool samples related to the first outbreak were not cultured for *Salmonella* spp., whereas culturing afterwards from both stored specimens and repeats, showed that some of these samples would have been positive for *S. enteritidis*. Thus without the application of stool culture rejection criteria the outbreak would have been detected one day earlier. With the four-day rule in effect, the outbreak might have been detected much later, if an unusually high number of nursing home residents with gastroenteritis had not been noticed by nursing home physicians. The rule was revised to prevent a possible delay in the future. As a result of this outbreak, the government has announced legislation forbidding the sale of *Salmonella*-contaminated eggs. An official ban on the use of raw eggs will be included in several hygiene codes.

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## Introduction

*Salmonella enterica* serovar *enteritidis* (*Salmonella enteritidis*) is a recognized cause of

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gastroenteritis associated with contaminated food, particularly poultry and eggs. According to a surveillance report by the Dutch Inspectorate for Health Protection and Veterinary Public Health in 1999, 0.03% of consumer eggs in the Netherlands were positive for *Salmonella* species.<sup>1</sup> Plans have been implemented to control and further reduce *Salmonella* contamination in poultry, preferably down to zero. However, as long as the risk of infection exists, both the Inspectorate and the Dutch Production Boards for Livestock, Meat and Eggs strongly advise against the use of raw eggs when preparing food for people in known risk groups (young, old, pregnant, immunocompromised).

The yield of enteric pathogens like *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Yersinia* spp. from stools of patients, who have been hospitalized for longer than three or four days, is minimal. Detection of pathogens or their toxins, other than *Clostridium difficile* is not considered worthwhile, except during a nosocomial food-borne outbreak.<sup>2-5</sup> The application of duration of hospital stay as a criterion for the rejection of stool cultures, like the 'four-day rule' used in our hospital, can result into substantial cost and time savings.<sup>6</sup>

Few authors have addressed this subject to date, therefore this study was undertaken to assess the delay in detecting a nosocomial outbreak caused by using a rejection protocol for stool cultures.

## Outbreak

The Isala klinieken is an 1100-bed, multi-site hospital, in Zwolle, The Netherlands. The Weezenlanden location comprises a hospital and a nursing home, both served by the same hospital kitchen.

On 11 October 2001, a nursing home physician reported several cases of diarrhoea to the clinical microbiologists. The symptoms of diarrhoea, together with nausea, vomiting and fever in about 30 residents, had apparently started on 8 October. The Municipal Health Service was informed and action was taken in accordance with the Guidelines for Institutional Outbreaks of Gastroenteritis, issued by the Dutch National Co-ordination Centre for Infectious Diseases. That same day, 11 October, stool samples of three of the patients were submitted for culture.

The next morning, all three cultures yielded suspect colonies that were identified within a few hours as *S. enteritidis* using Vitek 2 and by serotyping. Also on 12 October blood cultures from two septic clinical patients, at first thought not to be outbreak-related, became positive for

*S. enteritidis*, indicating a common source. An outbreak control team (OCT) was formed, and an investigation was started to assess the extent of the outbreak, to try to control it, and identify its possible source. By then about 40 people had fallen ill, nursing home residents as well as hospital patients and employees.

Anecdotal evidence focused attention on a strawberry bavaroise served as part of the noon meal on Sunday, 7 October. Two hospital employees, having only eaten the bavaroise, developed severe diarrhoea in the following days. This fact and well-known associations between bavaroise and salmonellosis made the dessert highly suspect.<sup>7</sup>

## Materials and methods

### Case finding and damage control

On 12 October, one of the infection control nurses together with one of the clinical microbiologists alerted all hospital and nursing home physicians, and attempted to identify all outbreak-related patients, using as a working case definition a patient with diarrhoea with onset of complaints between 7 and 12 October. Date of onset of diarrhoea and severity of illness were recorded for each symptomatic patient, if possible, and stool samples were collected for culture. The attending physicians and nurses were asked to report and culture every diarrhoea case showing in the days following.

All patients discharged after 7 October and all employees were advised by letter and telephone, respectively, to consult with their general practitioner (GP) or report to the hospital if symptomatic. GPs and medical institutions in the area, Isala clinical specialists and junior doctors were alerted to possible salmonellosis in Isala employees and discharged patients. All diarrhoea cases and most employees reporting themselves were cultured. All persons with symptoms of gastroenteritis were given 500 mg oral ciprofloxacin twice daily for five days.

### Microbiological and environmental investigations

From each patient one stool sample was cultured for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp. and *Escherichia coli* O157, and was tested for *C. difficile* toxin and rotavirus. Samples were additionally sent to the National Institute for Public Health and the Environment

(RIVM) to be tested for *Giardia lamblia*, *Cryptosporidium* spp. and Norwalk-like virus (NLV). If possible, stool samples from symptomatic patients submitted in the preceding days and rejected according to the four-day rule, were cultured for *Salmonella* spp. additionally.

For the culture of *Salmonella* spp. salmonella-shigella agar, xylose lysine deoxycholate agar, hektoen enteric agar and a Gram-negative enrichment broth were used. Suspect colonies were cultured on a triple sugar iron urea agar and tested for lysine decarboxylase. Final identification was accomplished using Vitek 2 (bioMérieux, Marcy l'Etoile, France) and serotyping, antimicrobial susceptibility was tested using an agar diffusion technique. Phage-typing of *S. enteritidis* strains was performed at the RIVM.

The hospital kitchen was inspected by Health Authorities, who paid special attention to the preparation method of bavaroise. Cultures were taken from eggs found in the kitchen and from the egg-producing farm (personal communication, Y.v.D.).

## Epidemiological analysis

October 7 was considered the most likely day of exposure, because of the bavaroise served on that day and the evidence of the two employees who had only eaten this dessert and fallen ill. Based on the fact that the first cases presented on 8 October and using the usual salmonellosis incubation period of 6-72 h,<sup>8</sup> a four-day exposure period, 6-9 October, was chosen for the analysis. A retrospective cohort study limited to hospital patients, who ate the noon meal on 6, 7, 8 or 9 October, was performed, as we expected a larger variation in choice of menu in this group as compared with nursing home residents. A case was defined as a hospital patient who ate the noon meal on 6, 7, 8 or 9 October, had onset of diarrhoea (three or more loose stools per day) during the period of 7-21 October and was culture-positive for *S. enteritidis*. Demographic data were obtained from the hospital computer system and the ordered menus from the Madows<sup>®</sup> food management system (Magister Nederland BV, Veldhoven, the Netherlands). An attempt to obtain food histories had to be aborted due to lack of staff. Food specific attack rates (AR) and percentage of exposed cases were calculated. Univariate data analyses were carried out using Epi Info version 6. Risk ratios (RR) with 95% confidence intervals (CI) were used to find associations between exposures and disease. Logistic regression using SAS version 8 was performed to adjust for confounding between different risk factors with RR > 2.0, both limits of

the CI > 1.0 and at least 11.5% of cases exposed. Odds ratios (OR) with 95% confidence intervals were calculated.

## Results

### Microbiology

In total 231 stool samples from nursing home residents, hospital patients and employees were cultured. Of these 82 (35%) were positive for *S. enteritidis* (Table I). Almost all hospital patients with diarrhoea were cultured (55/58). Five nursing home residents died during the outbreak, of which three could be cultured for *Salmonella*, yielding three positive results. Of these three one also had a positive blood culture. On 7 October, all five residents had strawberry custard for dessert.

By 21 October the outbreak was considered to be over, as no new cases emerged among hospital patients or residents and cultures were almost completed. The last stool culture was submitted on 23 October, by a hospital patient who developed diarrhoea after his discharge on 15 October. The exact date of onset of his symptoms was unknown. All cultures and tests for microbial agents other than *Salmonella* spp. were negative. All *S. enteritidis* strains were sensitive to ampicillin, trimethoprim/sulfamethoxazol and ciprofloxacin. Seven strains were phage-typed, all were pt 6.

Information about dates of onset of diarrhoea was available for 74% (61/82) of all *S. enteritidis* confirmed cases (Figure 1).

### Cohort study

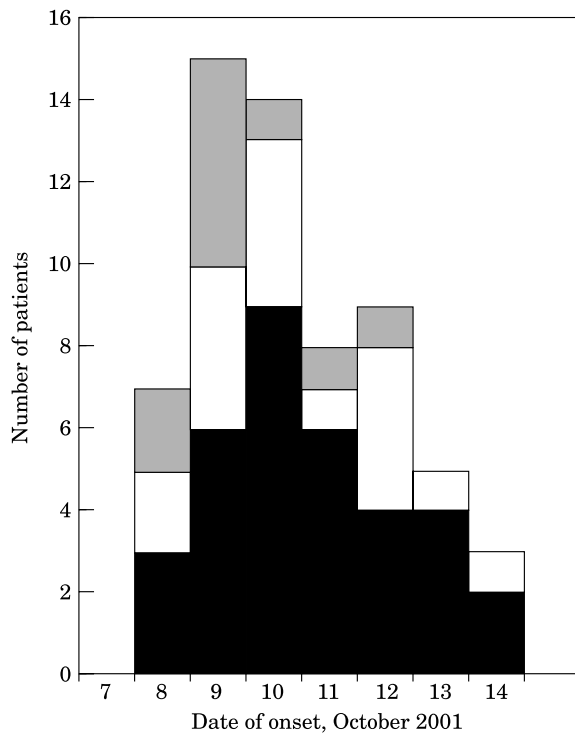
The cohort study included 421 hospital patients, of whom 51 (12.1%) were cultured because of diarrhoea. In total 26 (6.2%) cases were identified. Of the 169 different food items served in the period of 6-9 October, pork, pork gravy and strawberry

**Table I** Total number of patients cultured and number of *Salmonella enteritidis* positive patients in outbreak in the Netherlands, 2001

Patient group	Cultured (N)	<i>Salmonella enteritidis</i> positive (N)
Nursing home residents <sup>a</sup>	61	37 (61%)
Hospital patients <sup>a</sup>	55	26 (47%)
Employees <sup>b</sup>	115	19 (17%)
Total	231	82 (35%)

<sup>a</sup> Patients with diarrhoea.

<sup>b</sup> All employees that responded.



**Figure 1** Date of onset of diarrhoea of 74% of *Salmonella enteritidis* positive patients in outbreak in the Netherlands, 2001, among: ■, nursing home residents ( $N = 34$ ); □, hospital patients ( $N = 17$ ); ▒, employees ( $N = 10$ ).

custard on 7 October and julienne soup and beef gravy on 8 October showed stronger associations ( $2.0 < RR < 6.0$ ) with illness than the suspected bavaroise (Table II). Inquiries in the hospital kitchen revealed, that two days after preparation, the remainder of the bavaroise was added to the strawberry custard. Analysis of the two desserts combined, yielded the highest risk ratio in the univariate analysis (Table II). After logistic regression, the bavaroise-custard combination gave an OR of 7.6 (CI = 0.9-67.2), but pork, julienne soup and beef gravy were still associated

with disease. Of these, however, only the julienne soup and bavaroise-custard desserts could explain a high percentage of cases, 76.9 and 96.2%, respectively (Table II). Further research showed that all cases who had eaten julienne soup on 8 October also had eaten either bavaroise or custard on 7 October.

### Environmental investigations

Inspection of the hospital kitchen made clear that on 5 October bavaroise had been prepared by stirring raw egg yolk beaten with sugar into hot milk that had just been boiled with gelatine. After sufficient thickening the mixture was cooled to about 20°C, after which pasteurized egg white, sugar and whipped cream were added. During this process no temperatures were checked. The dessert was poured into disposable coupes that were refrigerated, together with the remainder of the bavaroise still in the bowl in which it had been prepared. The registered refrigerator temperature appeared to be too high. On 7 October the remainder of the bavaroise was added to the freshly prepared strawberry custard and both desserts were served. Cultures taken from eggs found in the kitchen and from the egg-producing farm were negative for *S. enteritidis* (personal communication, Y.v.D.). No leftover food samples were available for analysis.

### Discussion

In past outbreaks of *S. enteritidis* in Western Europe and the United Kingdom, the predominant phage-type isolated was phage-type 4.<sup>9</sup> Phage-type 6, another probably egg-associated phage-type, but thus far, a more rare cause of salmonellosis.<sup>10,11</sup>

**Table II** Food specific attack rates (AR), risk ratios, and adjusted odds ratios in *Salmonella enteritidis* outbreak in the Netherlands, 2001

Date	Food item	Eaten		Not eaten		Univariate analysis Risk ratio (95% CI)	Multivariate analysis Odds ratio (95% CI)	Cases expose (%)
		Cases	AR (%)	Cases	AR (%)			
Oct. 7	Pork	6	37.5	20	7.2	5.2 (2.4-11.1)	4.8 (0.9-25.8)	23.1
	Pork gravy	3	37.5	23	8.1	4.6 (1.7-12.3)	0.1 (0.0-1.7)	11.5
	Strawberry bavaroise	22	10.3	4	5.1	2.0 (0.7-5.6)	-	84.6
	Strawberry custard	3	30.0	23	8.9	3.7 (1.3-10.3)	-	11.5
	Bavaroise + custard	25	11.2	1	1.5	7.6 (1.1-55.0)	7.6 (0.9-67.2)	96.2
Oct. 8	Julienne soup	20	10.5	5	4.0	2.6 (1.0-6.8)	3.0 (1.0-9.0)	76.9
	Beef gravy	6	37.5	19	6.4	5.9 (2.7-12.7)	10.6 (1.8-63.7)	23.1

95% CI: 95% confidence interval.

Antibiotic therapy for all symptomatic persons was decided on as soon as the outbreak was confirmed, considering the number of people involved, the severe morbidity of many patients, and the fact that at the time three had already died. Oral ciprofloxacin was chosen for treatment because it significantly shortens the duration of clinical symptoms in patients with gastroenteritis and eradicates the causative organism successfully.<sup>12-16</sup> By shortening excretion of *Salmonella* spp. cross-infection and secondary spread can be prevented. The nursing home physicians felt the prompt administration of ciprofloxacin among severely ill nursing home residents contributed to limiting the mortality. The pattern of dates of onset of diarrhoea of all culture-positive patients suggested a point source and showed an incubation period of up to seven days.<sup>17</sup> This was consistent with the serving of contaminated strawberry bavaroise on 7 October, although the normal incubation period for *Salmonella* is 6-72 h (usually 12-36 h).<sup>8</sup> However, a prolonged incubation time has been described for *S. enteritidis* in other outbreaks, possibly due to a low dose of the infective agent in the contaminated food item.<sup>18,19</sup> Anecdotal evidence, known associations between bavaroise and salmonellosis, and the way of preparing the bavaroise served on 7 October, incriminated this dessert as the possible outbreak source from the start of investigations.<sup>7</sup> However, all five deceased nursing home residents chose strawberry custard for dessert on 7 October, and in the cohort study the custard showed a stronger association with illness than the bavaroise. This was explained by the fact that part of the bavaroise was added to the custard, as was reflected by the epidemiological analysis, which showed that eating either the bavaroise or the custard on 7 October formed a high risk of becoming ill and explained 25 of the 26 cases.

When nursing home residents develop symptoms of gastroenteritis, stool culture for *Salmonella* spp. is not routinely performed. The institutional kitchen serving them is supposed to work according to food hygiene standards and the system of hazard analysis of critical control points (HACCP) which was implemented. So, a food-borne nosocomial outbreak originating from this kitchen was considered unlikely. This means that an outbreak will not be easily recognized by the microbiology laboratory. In this case it was first suspected when during an informal meeting on 10 October, nursing home physicians discussed an unusually high number of residents with diarrhoea staying in different wings of the home.

The affected hospital patients too were distributed over many different wards. So, particularly in

the first days, in the hospital the outbreak escaped detection by hospital staff. Of two septic patients, blood cultures yielded growth on 12 October, identified as *S. enteritidis*. It is impossible to tell whether these results would have led to immediate recognition of the outbreak either in the laboratory or in the hospital, considering the two patients were in non-adjacent wards.

The number of stool samples from the hospital received by the laboratory increased as more people became ill, but this was not noticeable among the usual sample numbers. In the period between exposure and outbreak detection (7-12 October) stool samples of 21 hospital patients were submitted for culture. Of these, five were culture-negative. The other 16 were not cultured for *Salmonella* spp. in accordance with the four-day rule, i.e. stool samples from patients hospitalized for more than four days are rejected for culture of enteropathogenic bacteria other than *C. difficile*. This group of 16 contained 11 patients who were later confirmed to be outbreak-related. Culturing afterwards showed that nine would have been positive for *S. enteritidis* if cultured straight away. The first seven samples of outbreak-related symptomatic hospital patients arrived at the laboratory on 10 October. Of these, one was culture-negative, the other six were not cultured for *Salmonella* spp. Four of the six rejected samples were positive when cultured afterwards, to which end either stored specimens or repeat samples were used.

Suspect colonies in these four cultures on 11 October would probably have attracted attention and could have been identified by Vitek 2, with serotyping the same day. Therefore that without the application of the four-day rule, the outbreak would have been detected among hospital patients one day earlier. This probably would have affected the outcome in terms of mortality, because treatment of the severely ill patients could have begun one day earlier. With the four-day rule in place and without the warning from the nursing home physicians, the outbreak might not have been detected until much later than 12 October, with all due consequences. To help prevent a possible delay in the future, but wishing to keep the benefit of cost savings, we have modified the four-day rule in use according to the guidelines for stool culture proposed by Bauer *et al.*<sup>20</sup> They showed, that adding, for instance, an age criterion by which, irrespective of the duration of hospital stay, stools from elderly (>65 years) patients is not rejected for culture of enteric pathogens such as *Salmonella* spp., may help detect cases of sporadic nosocomial diarrhoea or nosocomial outbreaks earlier. Three of

the four above-mentioned rejected, but probably positive, stool samples were obtained from patients older than 65 years. Apart from this, an institutional kitchen serving vulnerable patient groups must follow strict food hygiene standards before any stool culture rejection criteria are applied. The use of egg yolk from uncooked shell eggs for preparing a bavaroise is strongly discouraged unless strict temperature monitoring during preparation and storage is performed. It appeared, during the inspection of the kitchen, that temperatures were not measured during essential heating and cooling steps and storage temperatures were too high.

Finally, this particular outbreak caused the Ministry of Health, Welfare and Sports to speed up and strengthen their prevention policy regarding *Salmonella*-contaminated eggs. In the near future, in the Netherlands, it will be unlawful to sell *Salmonella*-contaminated eggs for direct consumption. This includes imported eggs, for which the European Committee is notified. Besides, an official ban on the use of raw eggs during preparation of products for consumption that are not sufficiently heated (such as bavaroise and tiramisu) will be included in the food hygiene codes for health institutions, and hotel and catering industry.

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