Epidemiol. Infect. (1994), **112**, 235–245
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An outbreak of diarrhoeal disease attributed to *Shigella sonnei*

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(*Accepted 13 October 1993*)

SUMMARY

An outbreak of gastroenteritis occurred in a village on the Island of Crete, with 1479 inhabitants. One hundred and thirty-eight symptomatic patients from 57 different families were examined. Thirty percent of children under 12 years were affected compared with 4% of adolescents and adults ($P < 0{\cdot}0001$). Thirty-five out of 105 stool cultures (33%) grew *Shigella sonnei*. Thirty-four isolates had the same susceptibility pattern and were sensitive to ampicillin, while one was resistant to this antibiotic. Thirty-three isolates had the same plasmid profile. Based on epidemiologic and environmental findings, it was concluded that the transmission of shigellosis was waterborne. Shigella were not isolated from water, but one of the three village springs was found to be contaminated with faecal coliforms. This spring was probably contaminated by sewage material, due to its proximity to a source of untreated sewage effluent. Implementation of environmental and control measures brought the epidemic to an end.

INTRODUCTION

Shigellosis is a problem in both developed [1] and under-developed countries [2]. The countries of the eastern Mediterranean Sea are regarded as an endemic area of this disease [3], but there are few reports of its incidence and epidemiology in this region [3, 4]. Shigellosis is usually transmitted from person to person in households [5, 6], however outbreaks due to contaminated food [7–9] or water [6, 10] are not unusual. We report an outbreak of shigellosis in a Greek village on the island of Crete, which was linked to contaminated drinking water.

Background

Prophitis Elias is a village in the rural part of the Heraklion area of the island of Crete with 1479 inhabitants, 85% of whom are farmers. The village is located on a hill with valleys on each side. Although the history of the village dates back to the Byzantine era, and some construction from that time still exists, the

https://doi.org/10.1017/S0950268800057642 Published online by Cambridge University Press

majority of the houses are new and the water and sewage systems are approximately 20 years old. Each house has its own kitchen and bathroom with toilet, most of which are in good condition.

The water supply for the village is obtained from three fountains, (natural springs with holding tanks), two located on a hill higher than the village and a third located in the valley at a lower elevation than the urban area. This lower fountain has been found once previously to be contaminated by coliforms but no further investigation was made at that time. Water from the three fountains is collected by pumps in a central tank located on a hill higher than the village and then distributed to the houses.

Diarrhoeal illnesses are not uncommon in this area and occasional cases of salmonellosis have been reported in neighbouring villages during the previous 3 years.

On 26 December 1990 two children and an elderly woman from Prophitis Elias presented at the University Hospital of Crete at Heraklion complaining of diarrhoea, fever, abdominal cramps, headache, chills and malaise. *Shigella sonnei* was isolated from the stools of all of them. Isolates from the three patients had identical antibiotic susceptibilities by the agar diffusion method. The three patients did not belong to the same household and did not report common exposures such as group meals or social gatherings. However, they did report several other cases of diarrhoeal disease in the village.

On 27 December the local doctor and the mayor of the village notified the local Public Health Department of an escalating problem of diarrhoeal disease among the villagers, and that the majority of the patients did not seek medical treatment. They reported that on an empirical basis they had implicated the village's water supply and, on 24 December, took water samples for examination from the village's fountains and the central tank and then chlorinated the water in the tank. On the same date the valley fountain, which was known to have had some degree of contamination in the past, was isolated from the village's water supply network. From the beginning of the epidemic villagers residing in households with a case of diarrhoeal illness began drinking boiled or bottled water, because they empirically identified the water as the source of the problem.

METHODS

Epidemiologic and environmental investigation

On 28 December 1990 an investigation of the outbreak was begun in the village. A list of suspected cases was compiled from information supplied by the mayor, the local doctor, relatives and neighbours of the patients. Additionally, all villagers were instructed to contact the investigating team if they had experienced diarrhoeal disease. These names were added to the list of suspected cases.

The suspected cases were questioned about the day of onset of their disease, their symptoms, and the number of symptomatic relatives and healthy relatives in each household. A questionnaire was completed including the following information: food items consumed in the last 15 days, day care centre and school attendance, common meals in houses or parties, social gatherings, eating habits, Christmas celebrations attended, restaurants patronized, source of water supply

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at individual houses, and contact with another person with diarrhoea in the week before the onset of the illness. The condition of the patient's house and its location in the village, the condition of the house's water supply system, kitchen, bathroom, toilet, sewage system and the connection with the village's public sewage network were examined. Additionally, one stool sample for bacteriologic examination was collected from each individual who had experienced diarrhoea in the previous 2 weeks and presented for examination.

On 30 December, all three fountains and the central tank were inspected. Water samples from all of them were examined for coliforms and shigella. The plan of the sewage network was examined and all the locations where there was proximity to the water supply system were inspected. The terminal ends of sewage pipes were inspected as well.

Case definition

A case of shigellosis was defined as diarrhoeal disease with 5–6 bowel movements in a day for at least 2 days, with or without a stool culture positive for shigella.

A primary case was defined as the first case to develop signs and symptoms of shigella infection in each household. Cases in each household that developed within 48 h of the primary household case were designated as 'co-morbid cases'. Those cases, in each household, that developed 48 h or more following the primary cases were considered secondary cases. In tables and figures primary and co-morbid cases are combined for ease of presentation and are designated as 'primary cases'.

Statistical analysis

Differences between values of categorical variables were examined for statistical significance using Pearson's Chi Square test. Odds ratios and 95% confidence limits for these variables were estimated using the Mantel–Haenzel method. Continuous values were tested using the Mann–Whitney test.

Bacteriologic and plasmid DNA examination

Water samples were examined for coliforms and shigella as previously described [11].

Stool samples were obtained by using rectal swabs. The swabs were immediately introduced into Stuart's transport medium and brought to the laboratory within 1 h in cool conditions. The specimens were cultured by standard techniques for salmonella, shigella and campylobacter [12].

Antimicrobial susceptibility testing was performed by the agar diffusion method [13].

Plasmid profiles were examined by isolating plasmid DNA using an alkaline lysis method [14, 15].

RESULTS

Epidemiologic findings

One hundred and forty-eight villagers were considered suspected cases and interviewed. One hundred and thirty-eight persons, from 57 different households, who developed diarrhoeal disease between 11 December 1990 and 9 January, 1991

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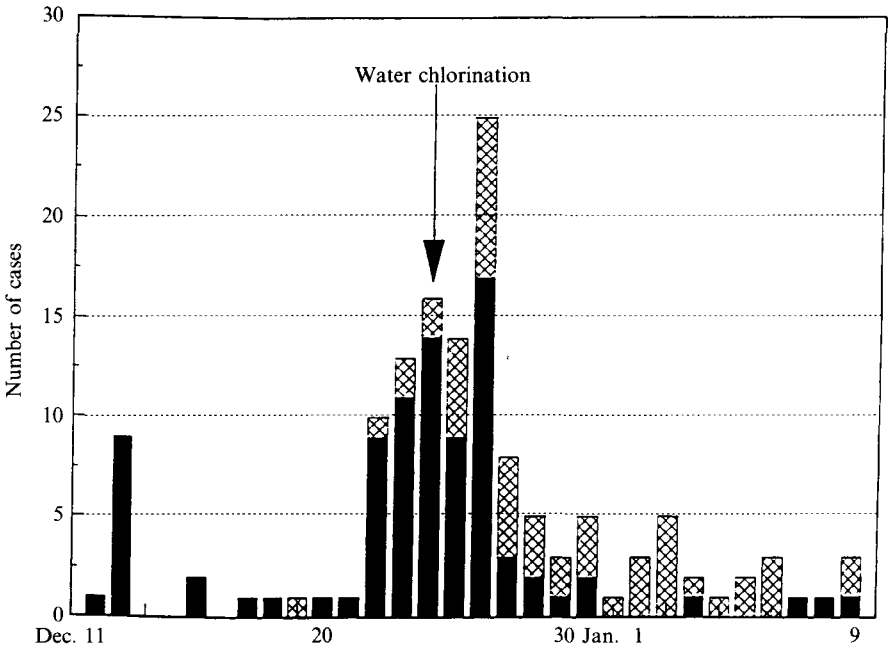

Fig. 1. Incidence of shigella cases. ■, Primary case; ⊠, secondary case.

were considered to have shigellosis. Rectal swabs were obtained from 105 of them and cultures were positive for *S. sonnei* in 35 (33%). Rectal swabs were unavailable for the remaining 33 villagers who met the clinical case definition. An additional ten persons developed gastrointestinal illness during the same time period but did not fit the case definition and were therefore excluded from the analysis. Cultures of rectal swabs taken from these individuals were all negative. From the information collected during interviews, it appears that at least another 60–70 people who became ill during the same time period did not contact the investigating team and were unavailable for examination.

All cases occurred between 11 December, 1990 and 9 January 1991 (Fig. 1). An initial peak of the epidemic occurred on 12 December with nine cases. After this date the epidemic continued with none, one or two cases daily until 22 December, when an escalation in the number of cases was observed with a minimum of ten cases daily, and reached a second peak on 26 December with 25 cases. Subsequently the epidemic tapered off with the last cases reported on 9 January. Secondary cases appeared first on 19 December with a peak on 26 December and they continued until the end of the outbreak on 9 January.

Seventy-seven patients were males and 61 females. Their median age was 10 years (range 5 months to 86 years). The clinical characteristics of the disease included diarrhoea (100%), abdominal cramps (74%), fever (54%), vomiting (36%), and headache (14%). Ten patients (7%) had bloody diarrhoea. The duration of symptoms ranged from 1 to 10 days (median 4). Children younger than 5 years of age had a longer duration of symptoms (median 5 days) when compared with adults (median 3 days). Only 20 out of 138 patients suffering from shigellosis

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Table 1. *Risk of developing shigella infection related to age*

Age (years)	Population at risk	No. of cases	Attack rate
< 5	100	23	23·0
5–12	196	66	33·7
13–18	180	11	6·1
> 18	1000	38	3·7
Total	1476	138	9·3
All ⩽ 12 years	296	89	30·1*
All > 12 years	1180	49	4·2*

* Odds ratio = 9·9 (95% CL: 6·7, 14·8); $P < 0·0001$.

Table 2. *Impact of age on the risk of developing primary or secondary shigella infection*

Age (yrs)	Primary cases		Secondary cases		Odds ratio	95% CL	P
	No.	%	No.	%			
< 5	18	18	5	5	4·2	(1·4, 13·5)	0·008
5–12	43	22	23	12	2·1	(1·2, 3·8)	0·01
13–18	7	4	4	2	1·8	(0·5, 7·4)	0·54
> 18	20	2	18	2	1·1	(0·6, 2·2)	0·87

sought medical attention. All of them received ampicillin in appropriate doses and their symptoms disappeared within 2–4 days.

The highest attack rates were observed in children between 5 and 12 years (33·7%) and the lowest in adults (3·7%). The overall attack rate in children under 12 years was 30% compared with 4% in adolescents and adults (OR = 9·9; CL = 6·7–14·8; $P < 0·0001$) (Table 1).

Fifty of the 57 households included 2 parents and 1 or more children younger than 18 years, and in each of these households at least 1 of the children was infected. In 32 of these households (64%), neither parents became ill, in 5 (10%), both parents became ill, in 4 (8%), the father became ill, and in 9 (18%) the mother became ill.

Children 12 years or less were significantly more likely to be primary cases than secondary cases whereas adolescents and adults were equally likely to be primary and secondary cases. The greatest difference was observed in children younger than 5 years who were four times more likely to be primary cases than secondary ($P = 0·008$) (Table 2).

Secondary transmission of shigella to household contacts was a major cause of morbidity and virtually all household contacts younger than 18 years developed infection by this route. In fact, among 37 household contacts younger than 18 years, only 4 children between 5 and 18 years and only 1 child younger than 5 years escaped infection. In contrast, 83 of 101 adult household contacts failed to develop infection ($P < 0·001$) (Table 3).

Because a majority of young household contacts were infected, it was not possible to evaluate the impact of the age of the primary case on the rate of secondary transmission. However, among adult household contacts, the rate of

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Table 3. *Risk of secondary transmission of shigella related to age of primary cases*

			Age of household contacts (years)							
			< 5		5–12		13–18		> 18	
Age of primary cases	Number of primary cases	Number of co-morbid cases*	Number at risk	Number of secondary cases	number at risk	Number of secondary cases	number at risk	Number of secondary cases	number at risk	Number of secondary cases
< 5	13	5	1	1	4	3	1	1	27	5
5–12	30	13	4	4	18	17	4	2	59	9
13–18	4	3	—	—	2	2	—	—	6	1
> 18	10	10	1	0	1	1	1	1	9	3
Total	57	31	6	5	25	23	6	4	101	18

* Co-morbid cases: cases in each household that developed within 48 h of the primary household case.

secondary transmission was 16% if the primary case was younger than 18 years, compared with 38% if the primary case was an adult ($P = 0{\cdot}15$). This finding should be viewed with caution due to the small number of individuals (nine) at risk in the adult primary case group.

One hundred and forty-eight persons answered the questionnaire regarding the possible source of the outbreak. All denied having eaten unusual or 'suspicious' food or common meals. Only 15 of them had eaten in 1 of the 3 restaurants of neighbouring villages during the week prior to the onset of symptoms. They had not changed their eating habits and no large social gatherings were reported despite the fact that the outbreak occurred during the Christmas holiday. None of the persons who developed the disease was a restaurant owner, food handler or professional cook. All had been drinking tap water from the village's supply system prior to the outbreak. However all started, on an empirical basis, drinking bottled or boiled water in households where a case of diarrhoeal illness occurred, because most of the villagers suspected that 'something was wrong with the water'. None of the people interviewed were aware of damage or leak in their house's sewage system or in the sewage system in their neighbourhood. All of them considered the water supply system of the village and within their houses to be in good condition.

Environmental findings

The 3 fountains (2 on the hill and 1 in the valley) and the central tank, where the water was gathered prior to distribution, were inspected and found in satisfactory condition.

Inspection of the sewage network of the village confirmed that it was in good condition and well functioning. However, the sewage pipes ended in four different locations at a distance of approximately 1000 m outside the outer limit of the village, in groves, where the sewage material was coming out unprocessed. One of those locations was found to be 6 m higher than the valley fountain at a distance of 600 m. Thus, unprocessed sewage material could have been carried by rain water towards the valley fountain.

Water specimens from the two hill fountains, the central tank and village house taps, collected before chlorination, on 24 December, did not reveal any coliforms, while water from the valley fountain revealed contamination by total coliforms (MPN 60/100 ml water) and faecal coliforms (MPN 20/100 ml water).

Shigella were not isolated from any water specimens collected from the contaminated valley fountain, the other two hill fountains, the central tank or the tap water from the village houses.

Bacteriologic findings

Thirty-four out of the 35 shigellae, isolated from the stool specimens, had the same sensitivity pattern. They were sensitive to ampicillin, cephalosporins, trimethoprim-sulfamethoxazole, tetracycline and chloramphenicol. One isolate was resistant to ampicillin.

Characterization of the *S. sonnei* isolates by plasmid profile showed three different plasmid patterns, as shown in Fig. 2. Thirty-three out of 35 shigella isolates had an identical plasmid profile. In lanes 3 and 5 of Fig. 2 are shown the

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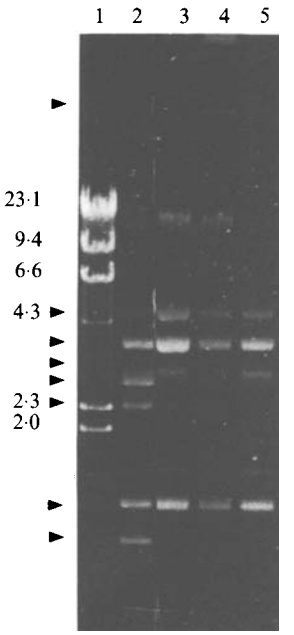

Fig. 2. Plasmid profile patterns of shigella isolates.

profiles of two of these isolates, with four major bands observed between 1·5 kb and 5 kb in each one. The remaining two isolates are shown in lanes 2 and 4. The isolate of lane 4 had the same profile as the 33 other isolates with the addition of a large size band. This isolate was resistant to ampicillin. The isolate of lane 2 had two major bands in common with the 33 isolates, while three small size bands are observed only in this isolate.

DISCUSSION

Based upon our epidemiologic investigation and on site inspection we have concluded that the outbreak of shigellosis in Prophitis Elias was waterborne. However, the organism was not isolated from water, including samples from the suspected fountain. This is not unusual in a waterborne outbreak [10, 11, 16]. Shigella is difficult to culture from water and may require samples of as much as 10 litres [6]. Nevertheless, the proximity of the sewage pipe end to the valley fountain, the run-off of the unprocessed sewage material towards this fountain, the contamination of its water with faecal coliforms, and the control of the outbreak after the isolation of this fountain and chlorination of the village's water supply, are strong indications of a waterborne outbreak. The epidemiologic findings revealed that at the beginning of the epidemic many cases of diarrhoeal disease appeared at the same time, in unrelated households, in different locations of the village, in persons with no other common exposures, indicating the water as the only likely source of the outbreak.

We were unable to identify the index case or cases responsible for the introduction of the shigella organisms in the village. Information about a teenager with severe diarrhoeal disease, visiting the village 20 days before the onset of the outbreak, could not be confirmed.

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Our data suggest that from the time the water became contaminated there was widespread transmission. Although contamination of food and person-to-person transmission is likely to have occurred, the waterborne route seems to have been the principal mode of transmission prior to the isolation of the contaminated fountain and chlorination of the water on 24 December. After this date person-to-person transmission is more likely, as suggested by the increased proportion of the secondary cases.

The total number of persons who became ill is not known, but according to the information available more than 200 people had symptoms of diarrhoeal disease between 11 December 1990 and 9 January 1991. We believe that a significant number of villagers with mild symptoms did not contact the investigators' team, since the majority of patients were reluctant to seek medical care. For the same reason, asymptomatic patients were not examined, although shigella infection can be present with mild or no symptoms at all [10, 11, 17]. Hence, we believe that a number of asymptomatic villagers could have been infected by shigella and spread the organism in their households and contacts, especially at school.

It can be assumed that a number of villagers had been exposed in the past to *Shigella* spp., since diarrhoeal diseases are not unusual in this area and the proximity of the fountain and the unprocessed sewage outlet had existed for years. Hence, it can also be assumed that a number of the people residing in the village who did not develop the disease probably had some degree of natural immunity to shigellosis, as described by other investigators [18].

The high attack rates observed in ages of less than 12 years reflect the inattention of children to hygiene and frequent physical contacts with other children of their ages. Similar high attack rates for this age have been reported [5, 10, 19].

Shigella was isolated from the stools of 33% of the examined persons. Earlier and repeated culturing would probably have uncovered more documented cases of infection. Optimal recovery rates typically require multiple, promptly taken cultures from both patients and asymptomatic household contacts [5, 20].

The bacteriologic findings suggest that the majority of the patients were infected by the same strain which was sensitive to ampicillin. Only one isolate was resistant to this antibiotic. The emergence of resistance is a possible explanation since the organism was isolated from a patient receiving ampicillin for one week [21, 22].

The plasmid profiles of 33 isolates were identical indicating that these organisms belonged to a single strain. The differences observed in the two remaining isolates are not necessarily indicators of the presence of other strains in this outbreak, since these differences are not considered major ones and are observed only in two isolates. A limited number of plasmids can be lost or acquired, making it difficult to use a strictly defined plasmid profile as the indicator of a single strain [23]. We speculate that the large size plasmid of the isolate of lane 4 may be responsible for the ampicillin resistance observed in this organism.

Shigellosis is linked to levels of personal and environmental hygiene [24]. This outbreak was the result of a severe negligence in the village's environmental hygiene which existed for years.

Epidemics of shigellosis can be controlled solely by implementation of

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environmental control strategies without the use of antibiotics [25]. This outbreak was controlled after proper environmental control measures had been taken, despite the fact that most individuals did not seek medical attention.

This study underscores the importance of environmental hygiene, supports the evidence that sewage polluted water can be a route of transmission of shigellosis and confirms that implementation of control measures can promptly end a shigella epidemic without the use of antibiotics.

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