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Two epidemics of diarrhoeal disease possibly caused by *Plesiomonas shigelloides*

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SUMMARY

Two epidemics of water-borne diarrhoeal disease involving a total of 1000 persons are reported. In both epidemics, none of the usual bacterial enteropathogens were recognized and *Plesiomonas shigelloides* was the only suspect aetiological agent isolated. The ecology of *P. shigelloides* was investigated in these outbreaks. It was recognized as an inhabitant of fresh surface water and its presence was closely related to warm weather.

INTRODUCTION

Although its role as an enteropathogen has not been conclusively proved, isolation of *Plesiomonas shigelloides* from patients suffering from diarrhoea has been reported by many workers (Schmid, Velaudapillai & Niles, 1954; Osada & Shibata, 1956; Vandepitte, Ghysels, Goethem & Marrecau, 1957; Ueda, Yamasaki & Hori, 1963; Aldova, Rakovsky & Chovancova, 1966; Geizer, Kopecky & Aldova, 1966; Hori *et al.* 1966; Cooper & Brown, 1968; Pauckova & Fukalova, 1968; Winton, 1968; Sakazaki *et al.* 1971; Zajc-Satler, Dragas & Kumelj, 1972; Bhat, Shanthakumari & Rajan, 1974; Jandl & Linke, 1976). Recently, two epidemics of diarrhoeal disease possibly caused by these organisms were encountered. We describe here the bacteriological and epidemiological findings in these outbreaks as well as the results of ecological studies on the presence of *P. shigelloides* in the environment.

MATERIALS AND METHODS

Stool specimens

Stools from patients were examined for bacterial pathogens including *Salmonella*, *Shigella*, enteropathogenic *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus* and *Clostridium perfringens* by conventional bacteriological methods. Briefly, faecal specimens were inoculated onto Salmonella-Shigella (SS) agar, mannitol-lysine-crystal violet-brilliant green (MLCB) agar, deoxycholate-hydrogen sulphide-lactose (DHL) agar, thiosulphate-citrate-bile salt-sucrose (TCBS) agar, *Clostridium welchii* (CW) agar, selenite-brilliant green (SBG) broth and salt-polymyxin (SP) broth. After overnight incubation, subcultures were made from SBG

broth to MLCB and DHL agar and from SP broth to TCBS agar. All cultures except for CW agar were incubated aerobically at 37 °C. All culture plates were examined after incubation overnight as well as after 48 h.

Water

Tap water from the Youth Activity Centre where the first epidemic occurred and samples of water and mud from the water cleaning facilities at the Centre were investigated for enteropathogens. Batches of ten samples of 100 ml of water were each filtered through 0·45 μm Millipore membranes and the filters then placed on 5 plates of SS and DHL agar respectively. All samples of mud (50 g) were inoculated into nutrient broth from which subcultures were carried out after overnight incubation onto the same media used for water samples.

In the ecological studies, which were carried out during three periods between August 1973 and December 1974, samples of water, mud and various animals inhabiting ponds, rivers and streams were collected in Toyono where the Youth Activity Centre is located, as well as from other areas in the Osaka district. Water and mud samples were examined as already described. Animals obtained from waters were homogenized and the whole material inoculated into nutrient broth. Only modified SS agar, in which 1 % inositol was substituted for lactose, was used in these ecological studies.

Identification of organisms isolated was performed according to the methods described by Sakazaki *et al.* (1971) and by Cowan (1974). Determination of the O and H antigens of *P. shigelloides* was performed as described by Shimada & Sakazaki (1977).

RESULTS

Clinical, epidemiological and bacteriological observations

Epidemic 1

Between 5 and 13 August 1973, an outbreak of diarrhoea occurred at the Youth Activity Centre of Toyono in Osaka. Of 2141 persons who stayed there between 5 and 10 August, 978 suffered from acute diarrhoea. The main clinical effects were diarrhoea (88 %), abdominal pain (82 %), fever (22 %) and headache (13 %). In most patients with fever the body temperature ranged from 37 to 38 °C. In the majority of cases, the stools were watery and occasionally mucous. Most patients recovered within 2 or 3 days and no secondary cases occurred in any family contacts. Epidemiological evidence did not incriminate any foods as the cause of the epidemic, and tap water was the only common factor noted.

Stool specimens from 124 patients on 12 and 13 August were obtained for bacteriological study. On investigation, none of the accepted pathogens such as *Salmonella*, *Shigella*, enteropathogenic *E. coli*, *Y. enterocolitica* and *V. parahaemolyticus* were detected, nor was *C. perfringens* incriminated. Of the 124 specimens, however, 21 which were obtained during the acute phase of diarrhoea yielded abundant growth of colourless translucent colonies resembling those of *Shigella* after overnight incubation on SS agar. Biochemical characterization of these cultures confirmed their identity as *P. shigelloides*. Serologically, they represented 3 different serovars, O17:H2 (16 strains), O22:H3 (4 strains) and O8:H5 (1 strain).

One of 8 samples of tap water, taken from different parts in the area on 11 August, yielded *P. shigelloides*. The serovar of this isolate was O17:H2, the same as the predominant type isolated from patients. In addition, *P. shigelloides* was also isolated from 4 of 12 water and 2 of 2 mud samples obtained from the water treatment plant in the area during August. Three of these 6 belonged to serovars O17:H2, 022:H3 and O2:H1a, 1c respectively, but the remainder were untypable.

Epidemic 2

On 21 October 1974, another outbreak occurred among employees at an electrical instrument company in Moriguchi City near the central area of Osaka prefecture. They had been on a sightseeing tour between 18 and 20 October and became ill after returning home. Twenty-four out of 35 participants suffered from acute diarrhoea on 21 and 22 October.

The main clinical features were diarrhoea (92 %), lassitude (63 %) and abdominal pain (54 %), fever did not occur in most patients. Diarrhoea was generally mild, watery in nature, sometimes with mucus and the frequency 3 or 4 stools a day. The duration of illness was 2–3 days. No particular food was suspected on epidemiological grounds.

Faecal specimens from eight patients were available for examination on 23 October. *P. shigelloides* was isolated from three of these specimens, virtually in pure culture. Each strain belonged to the serovar O24:H5.

Ecological investigations

During the period of the study, a total of 342 samples of water and mud were collected from ponds, rivers and shallow streams in Toyono and other areas. *P. shigelloides* was isolated from 132 (38·6 %) of these samples. The results are shown in Table 1. The organism was isolated more frequently from ponds than from other sources. Also, more isolations were obtained from mud than from water on all occasions. In general, the rate of isolation was higher in the warmer than in the cold seasons.

Several indigenous animals, including 17 fish, 6 shellfish and 5 newts, were also examined for the presence of *P. shigelloides* and it was isolated from 5, 4 and 4 of them respectively. The incidence of the organism was thus higher in newts and shellfish which live in mud than in fish and in water.

Table 2 shows the distribution of serovars among the strains of *P. shigelloides* isolated from patients and from the water and mud samples as well as the animals. Of 175 strains isolated, 118 represented 29 different serovars and the remaining 57 were untypable with the recognized O and H antisera of the species. The predominant serovars were O25:H3, O2:H1a, 1c, O9:H2, O27:H3, O22:H3 and O8:H3. No significant differences in the distribution of serovars in different areas were recognized. However, serovar O17:H2 which was predominant in epidemic 1 was isolated only in the Toyono area during the epidemic, and the single serovar O24:H5 found in epidemic 2 was not isolated in the ecological study.

Table 1. *Isolation of* Plesiomonas shigelloides *from water and mud*

Source		Toyono 1973 Aug.	Sept.	Nov.	Toyono 1974 June	July	Aug.	Sept.	Oct.	Dec.
Pond	Water	11/19*	1/1	2/4	3/3	3/4	4/4	4/4	0/4	0/4
	Mud	13/16	—	—	3/3	3/4	2/4	4/4	3/4	1/4
Shallow Stream	Water	4/39	1/1	0/1	0/8	0/9	1/9	0/9	0/9	0/9
	Mud	13/18	—	—	1/8	4/9	4/9	2/9	0/9	0/9
River	Water	3/4	—	—	0/1	1/1	0/1	1/1	1/1	1/1
	Mud	1/1	—	—	0/1	1/1	0/1	1/1	1/1	1/1
Total		45/97	2/2	2/5	7/24	12/28	11/28	12/28	5/28	3/28

Source		Other areas 1974, June	July	Oct.	Nov.	Dec.	Total
Pond	Water	4/5	4/5	3/3	—	0/5	39/65
	Mud	2/4	1/4	1/1	—	5/5	38/53
Shallow Stream	Water	—	—	2/6	—	—	8/100
	Mud	—	—	0/3	—	—	24/74
River	Water	1/6	0/2	2/4	0/2	0/4	10/28
	Mud	3/6	1/1	1/4	1/2	2/2	13/22
Total		10/21	6/12	9/21	1/4	7/16	132/342

* Denominator indicates the number of samples examined. Numerator indicates the number of samples positive for *Plesiomonas shigelloides*.

Table 2. *Serovars of* Plesiomonas shigelloides *isolated from water, mud and animals*

Serovar O	Serovar H	Number of strains	Serovar O	Serovar H	Number of strains
2	1a, 1c	14	22	3	10
2	7	1	22a, 22c	3	2
3	2	1	23	1a, 1c	1
3	3	1	23	3	1
4	3	6	25	3	17
6	3	1	25	6	1
7	2	1	26	1a, 1c	4
7	5	1	27	2	5
8	2	2	27	3	11
8	3	9	3	UT†	1
8	5	1	7	UT	1
9	2	12	10	UT	1
10	2	1	12	UT	2
12	3	1	25	UT	1
14	5	1	UT	1a, 1c	15
17	2	5	UT	2	18
18	3	2	UT	3	14
18a, 18c	3	3	UT	10	1
18a, 18c	NM*	1	UT	UT	3
19	2	2			

* Nonmotile † Untypable.

DISCUSSION

Although the aetiological role of *P. shigelloides* is uncertain, its presence in stools of patients with diarrhoea of otherwise unexplained origin has been reported by many investigators. In the two epidemics described here, *P. shigelloides* possibly had some aetiological significance since it was mostly isolated from the stools of patients in the acute stage of the disease and since no other bacterial enteropathogens were recognized. In epidemic 1, *P. shigelloides* was isolated from only 16·9% of all the stool specimens examined. Though this isolation rate may seem low if the organism is regarded as the aetiological agent, the majority of the 124 specimens studied were, however, from convalescent patients since notification of the epidemic, which had begun on 5 August, was received in the office of the Public Health Service after the peak of the event.

Aldova *et al.* (1966), Geizer *et al.* (1966), Cooper & Brown (1968) and Zakhariev (1971) suggested that *P. shigelloides* may be water-borne. The findings in the present study also support this suggestion. In the district where epidemic 1 occurred, water was pumped up from a pond to the water treatment plant and then filtered and chlorinated. As mentioned, the serovar (O17:H2) of the organisms mostly found in patients was also detected in tap water, as well as water and mud in the pond. The fact that the organism was isolated from tap water suggests that chlorination of the water was unsatisfactory. Although both epidemics of *P. shigelloides* infection were thought to be associated with drinking water, it seems unlikely that this was the direct vehicle of transmission since, like other aetiological agents such as *Salmonella* and *V. parahaemolyticus*, large numbers of organisms are usually needed to cause illness. It is more likely that *P. shigelloides* would multiply and reach infective numbers after water-borne transmission to various foods.

It is clear that *P. shigelloides* is an inhabitant of surface water. It is of interest that the ecology of this organism is perhaps analogous to that of *V. parahaemolyticus* in coastal waters. The environmental investigations suggest that the presence of *P. shigelloides* in water is perhaps more frequent in the warmer season than in the winter season. Though many natural water bacteria are psychrophilic the optimum temperature for growth of *P. shigelloides* ranges from 30 to 35 °C. This range is similar to that reported for *V. parahaemolyticus* (Sakazaki, 1969). More isolations of the organism were obtained from mud than from water on all occasions. This finding perhaps suggests that the optimum place for survival and growth of this organism may be in mud which presumably contains sufficient nutrients. A similar suggestion was made for *V. parahaemolyticus* by Kaneko & Colwell (1973).

With regard to enteropathogenicity, many investigators consider that *P. shigelloides* can cause diarrhoea in man. Ligated ileal loop tests in rabbits with this organism have, however, so far given negative reactions (Sanyal, Singh & Sen, 1975; Tamura & Sakazaki, unpublished) nor do they produce enterotoxin(s) such as those of *Escherichia coli* (Shimada *et al.* unpublished). Further studies are needed to define the role of this organism in the production of diarrhoeal disease.

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