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A survey of *Plesiomonas shigelloides* from aquatic environments, domestic animals, pets and humans

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SUMMARY

We conducted a survey during the period from 1974 to 1976, to determine the distribution of *Plesiomonas shigelloides* in human faeces, the intestinal contents of cattle, swine, poultry, dogs, cats, fresh water fish, and river water and sludge from wet riverbeds in the vicinity of Tokyo. Isolation of the organisms was performed by using Salmonella-Shigella (SS) agar and deoxycholate-hydrogensulphide-lactose (DHL) agar plates.

P. shigelloides was isolated from 3 (0·0078%) of 38454 healthy Tokyoites, 37 (3·8%) of 967 dogs, 40 (10·3%) of 389 cats, 25 (10·2%) of 246 fresh water fish, 64 (12·8%) of 497 river water samples, and 2 of 19 (10·5%) sludge samples.

Of 302 strains isolated, from dogs, cats, fresh water fish, river water and healthy carriers, 196 were typed to 50 serovars. Most of the serovars were found to be similar to strains isolated from patients with gastroenteritis due to *P. shigelloides*.

INTRODUCTION

Plesiomonas shigelloides was first isolated from the faeces of a patient for whom no clinical history was available. It was reported by Ferguson and Henderson (1947) to be a motile organism with the major antigen of *Shigella sonnei* phase I, and named Paracolon C27. Biochemical, morphological and taxonomical examinations of the organism were performed by many investigators and the organism was classified as a new genus *Plesiomonas,* and named *Plesiomonas shigelloides* (Habs & Schubert, 1962).

P. shigelloides was frequently sporadically isolated from patients with diarrhoea and sometimes from healthy individuals (Sakazaki *et al.* 1971; Cooper & Brown, 1968; Geizer, Kopecky & Aldova, 1966) and a connexion between diarrhoeal

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disease and the presence of large numbers of the organisms in the faeces was suspected. However, no definite conclusions on the pathogenicity of the organism have been reached to date (Sakazaki *et al.* 1959).

There have been several reports by Japanese authors on outbreaks of infectious gastroenteritis in humans due to *P. shigelloides* (Hori *et al.* 1966; Tsukamoto *et al.* 1978; Ueda, Yamasaki & Hori, 1963). However, the source(s) of these infections have rarely been discovered and to our knowledge, no ecological survey on the organisms has been performed.

Therefore, the present study was conducted to determine the distribution of *P. shigelloides* in human faeces, the intestinal contents of cattle, swine, poultry, dogs, cats, and fresh water fish; and in river water and sludge from the vicinity of Tokyo.

MATERIALS AND METHODS

The materials examined in the present study were collected in the vicinity of Tokyo from 1974 to 1976.

River water

River water samples were collected once or twice a month at 20 sites along the Tama River (Fig. 1). Additional samples were taken from other sites along the same river. The river water (500 ml) was collected in sterilized bottles and filtered through membrane filters (Millipore Corp., type HA, 0·45 μm). The filter disk was stamped directly onto 10 plates of Salmonella-Shigella (SS) agar and deoxycholate-hydrogensulphide-lactose (DHL) agar, successively.

Sludge

Nineteen sludge samples from the wet riverbeds and aquatic sites of the Tama River were examined. Each sludge sample (100 g) was thoroughly mixed with 300 ml of sterile saline. After a few minutes, the supernatant fluid was removed to another glass. A 0·1% Potassium aluminium sulphate solution was added to make a $1/10^6$ concentration, and 10% NaOH solution was added to adjust the pH to 6. The supernatant fluid was then decanted and a drop of the precipitate was plated onto 10 plates of SS agar and DHL agar, successively.

Fresh water fish

Near the sampling sites of the Tama River, 169 fresh water fish were caught In addition, during the summer months, 77 fresh water fish were caught in the Naka River. The whole intestine of each fish was mixed with 5 ml of saline, and homogenized specimens were plated onto 3 plates of SS agar and DHL agar.

Cattle, swine and poultry

The intestinal content of 105 cattle, 310 swine and 98 poultry, obtained from local abattoirs, were cultured.

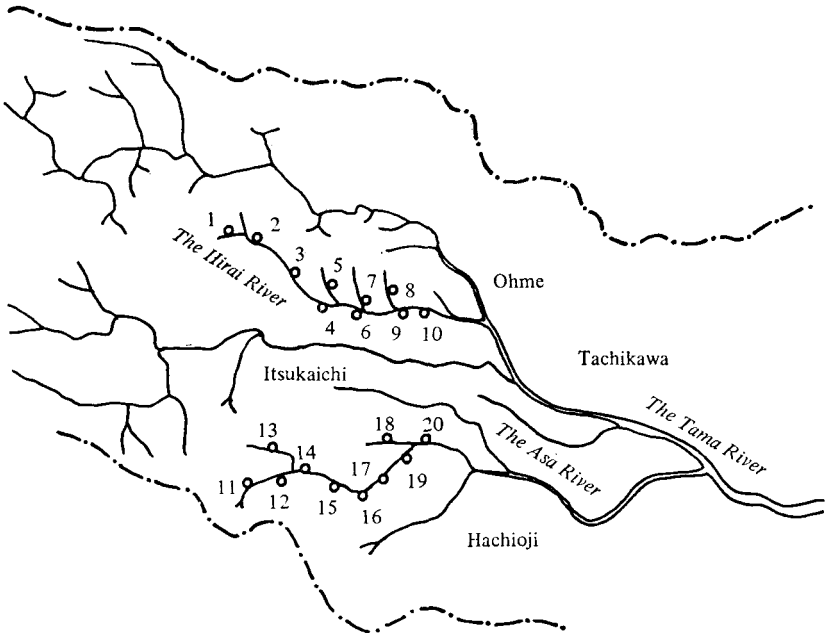

Fig. 1. Sites where the river water was collected.

Dogs and cats

The intestinal content and mesenteric lymph nodes of 967 dogs and 389 cats caught within the Tokyo area, or for which euthanasia was requested by the Tokyo Metropolitan Dog Pound Office, were cultured.

Humans

A total of 38454 healthy subjects, including school children and food handlers, was examined.

SS agar and DHL agar plates were incubated at 37 °C for 18–24 h. Suspected colonies were inoculated in triple-sugar-iron (TSI) agar and lysine-indole-motility (LIM) medium. The organisms that produced alkaline slants, acid butts without gas in TSI agar, lysine decarboxylase, and indole in LIM medium, were further examined, using biochemical tests according to the method of Ewing, Hugh & Johnson (1961).

Serological typing

Serological typing of all strains suspected of being *P. shigelloides* was done by the methods of Shimada & Sakazaki (1978). For O antigen determination, tube agglutination tests were carried out with heated cell suspension and the results were read after incubation in a water bath at 50 °C for 18 h. For H agglutination, live cultures of actively motile organisms in brain-heart infusion broth were used and the results were read after incubation at 56 °C for 6 and 18 h.

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RESULTS

Isolation from river water, sludge and fresh water fish

Of a total of 350 river water samples obtained from 20 sampling sites in the Tama River, 31 (8·9%) collected at 15 sampling sites, were positive for *P. shigelloides*. Table 1 shows that the organisms could only be isolated in samples collected during the period from July to November. Of 147 water samples collected from other sites in the Tama River, 33 (22·4%) were *P. shigelloides* positive, as were 2 of 19 (10·5%) sludge samples from the wet riverbed. The organism was found in 25 (10·2%) of 246 fresh water fish caught in the Tama and Naka Rivers. Fish caught from July to September in the Tama River were *P. shigelloides* positive, while those caught from February to June were negative (Table 2).

Isolation from animals and humans

We could not isolate the organism from 659 specimens of the intestinal contents of cattle, swine and poultry. These samples were independently examined over short periods; for example cattle were examined between May and June, swine between November and December and poultry in February.

Dogs and cats were carriers of the organism. Of 967 dogs, 37 (3·8%) were positive. The organisms were isolated from the caecal content of 14, the rectal content of 8, the mesenteric lymph nodes of 10, and from both the caecal and rectal contents of 5 dogs. Of 389 cats, 40 (10·3%) were carriers. The organisms were isolated from the caecal contents of 6, the rectal contents of 31, the mesenteric lymph nodes of 1, and from both the caecal and rectal contents of 2 cats (Table 3). Most of the carriers were symptomless.

Of the 38454 healthy humans examined, *P. shigelloides* was identified in 3, two adults and one child. These carriers were found only in the summer season.

Serological types of P. shigelloides *isolated*

For epidemiological studies, all strains isolated were typed by the methods of Shimada & Sakazaki (1978) who studied the serovars of the organisms and defined 30 O antigen groups, including some somatic antigen groups also found in the genus *Shigella*, and 11 H antigens (Table 4). Of 53 strains isolated from 37 dogs, 33 were typed to 14 serovars, 11 were determined as only O antigens, and 9 were determined as only H antigens. The predominant serovars of the cultures from dogs were O17:H2 (8) and O1:H5 (4). Of 59 strains isolated from 40 cats, 39 were typed to 11 serovars, 16 were determined as only H antigens and 4 as neither O nor H antigens. The predominant serovars of the cultures from cats were O3:H2 (11), O19:H2 (9) and O18:H2 (5). Occasionally, some cultures from the same dog or the same cat were typed to more than one serovar.

Of 36 strains isolated from 25 fresh water fish, 20 were typed to 14 serovars, 4 were determined as only O, 11 as only H, and one as neither O nor H antigens.

Of 149 strains isolated from 64 samples of river water, 102 were typed to 35 serovars, 41 were determined as only H and 6 as neither O nor H antigens. The

Table 1. *Detection of* Plesiomonas shigelloides *in water from the Tama River*

Month	Sampling sites 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Jan.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Feb.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mar.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Apl.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
May	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
June	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
July	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aug.	–	+	–	+	+	+	–	+	+	+	–	+	–	+	+	–	–	–	+	+
Sep.	+	+	–	–	+	–	–	+	+	+	–	–	–	+	+	+	–	–	–	–
Oct.	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	+	–
Nov.	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Dec.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 2. *Detection of* Plesiomonas shigelloides *in fresh water fish*

Location collected	Species of fish	No. examined	No. positive
The Tama River	Pale chub	68	5
	Japanese fatminnow	45	0
	Field gudgeon	41	12
	Others	15	2
	Sub total	169	19 (11·2%)
The Naka River	Crusian carp	72	5
	Carp	4	0
	Other	1	1
	Sub total	77	6 (7·8%)
Total		246	25 (10·2%)

Table 3. *Detection of* Plesiomonas shigelloides *in the intestinal contents and mesenteric lymph nodes of dogs and cats*

Species	No. examined	No. positive	Specimens: Caecal contents	Rectal contents	Mesenteric lymph nodes	Total
Dogs	967	37	+	–	–	14
			–	+	–	8
		(3·8%)	–	–	+	10
			+	+	–	5
Cats	389	40	+	–	–	6
			–	+	–	31
		(10·3%)	–	–	+	1
			+	+	–	2

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Table 4. *Serovars of* Plesiomonas shigelloides *isolated from dogs, cats, fresh water fish, river water and healthy carriers*

O antigen		H antigen	Number of strains					
			Dogs	Cats	Fresh water fish	River water	Healthy carrier	Total
1	:	1	—	—	—	1	—	1
1	:	2	1	—	—	4	—	5
1	:	5	4	—	—	—	1	5
2	:	1	—	2	1	15	—	18
2	:	2	1	—	—	—	—	1
2	:	3	—	—	—	1	—	1
2	:	5	—	—	—	5	—	5
3	:	2	1	11	—	3	—	15
3	:	3	—	—	—	3	—	3
3	:	5	—	—	1	—	—	1
4	:	3	—	—	3	7	—	10
5	:	3	—	—	—	1	—	1
6	:	1	—	—	—	1	—	1
6	:	3	—	—	2	—	—	2
7	:	2	—	—	1	—	—	1
8	:	2	2	1	1	—	—	4
8	:	3	—	—	3	10	—	13
10	:	3	—	—	—	2	—	2
11	:	2	—	—	—	1	—	1
11	:	3	—	—	—	2	1	3
12	:	3	—	—	—	2	—	2
12	:	5	—	—	—	2	—	2
13	:	2	1	—	—	—	—	1
14	:	2	1	—	—	—	—	1
15	:	2	—	1	—	—	—	1
16	:	5	—	—	1	1	—	2
17	:	2	8	—	—	—	—	8
17	:	11	2	—	—	—	—	2
18	:	2	—	5	—	2	—	7
19	:	2	3	9	—	3	—	15
19	:	3	2	—	—	—	—	2
20	:	10	—	—	—	1	—	1
21	:	2	1	—	—	1	—	2
22ab	:	1	—	—	—	1	—	1
22ab	:	2	—	—	1	1	—	2
22ab	:	3	—	—	1	4	—	5
22ac	:	3	—	—	1	1	—	2
23	:	1	—	1	1	1	—	3
24	:	3	—	—	—	1	—	1
25	:	3	—	—	2	7	—	9
26	:	1	—	—	—	7	—	7
26	:	5	—	—	—	1	—	1
27	:	1	—	—	—	3	—	3
27	:	3	—	2	—	3	—	5
27	:	5	—	—	—	1	—	1
28	:	3	3	—	—	1	—	4
29	:	2	—	1	1	2	—	4
29	:	3	—	3	—	—	—	3
30	:	1	—	3	—	—	—	3
30	:	2	3	—	—	—	—	3
Sub total			33	39	20	102	2	196
Untypable			20	20	16	47	3	106
Total			53	59	36	149	5	302

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predominant serovars were O2:H1 (15), O8:H3 (10), O25:H3 (7), O26:H1 (7) and O4:H3 (7).

The 5 strains isolated from 3 healthy carriers were typed to 2 serovars. Two strains were determined as only O or H antigens, and 1 as neither O nor H antigens.

Biochemical characteristics

The biochemical characteristics of the strains were very similar to those reported by Ewing *et al.* (1961). However, our results differed from theirs with respect to ornithin decarboxylase. All of the strains we isolated were positive, while 45% of the strains reported by Ewing *et al.* (1961) were positive.

DISCUSSION

We performed an ecological study of *P. shigelloides*. As this organism resembles genus *Aeromonas* isolated from river water and fresh water fish (Ewing *et al.* 1961; McCarthy & Rawle, 1975), river and fresh water fish from the Tama River and its tributaries, the Hirai and Asa Rivers, and fresh water fish from the Naka River were examined. Our findings confirmed that the organism is distributed in river and fresh water fish. Furthermore, we found the river and fresh water fish to be widely and strongly contaminated by the organisms in the summer season (Tables 1, 2).

It has been suggested that the river and fish contamination may be due to excretions of domestic animals or sewage from slaughterhouses. We examined the intestinal content of cattle, swine and poultry and found them to be negative for *P. shigelloides*. Domestic animals examined during the period from November to June appeared to be scarcely contaminated by the organism. This suggests that the contamination of animals depends to a high degree on their habitat (Schmid, Velaudapillai & Niles, 1954).

Dogs and cats, on the other hand, were found to be strongly contaminated by the organism throughout the year. The carrier rate was 3·8% for dogs and 10·3% for cats (Table 3).

Many of the cultures from dogs and cats were of the same serovars as those from diarrhoeic patients (Shimada & Sakazaki, 1978), for example, O3:H2, O13:H2, O17:H2, O19:H2, suggesting that dogs and cats are important carriers of the organisms and may play a role in human infections.

During this survey, we isolated the organism from a dog excreting bloody faeces. The serovars of the isolates were O21:H2, O8:H2 and O17:H2, suggesting that the organism could be a common pathogen in dogs and humans.

Strains isolated from river and fresh water fish have the same serovars as those from diarrhoeic patients (Shimada & Sakazaki, 1978), for example, O2:H1, O4:H3, O8:H3, O22:H3, O25:H3. From the viewpoint of serological coincidence, river water may be important as a source of human infection. Most of the infectious gastroenteritis outbreaks in humans caused by this organism occurred during the summer season, a time which corresponds to the environmental contamination, as shown by our ecological surveys. Tsukamoto *et al.* (1978) reported a mass infection

in children during the summer, which was caused by *P. shigelloides*. The source was traced to tap water derived from a pond.

Most of the humans examined in the present study were adults. Of 38454 healthy Tokyoites, 3 (0·0078%) were carriers, two of these were adults and one was a child. Geizer *et al.* (1966) reported isolating *P. shigelloides* from one faeces sample (0·13%) of 756 children up to the age of six years. These results suggest that the carrier rate of children may be higher than that of adults. In the mass infection reported by Hori *et al.* (1966), the infection rate of children under 15 years of age was higher than that of adults. In addition, the children's illness was frequently complicated by fever. Based on the symptoms reported by Hori *et al.* (1966) and Tsukamoto *et al.* (1978), children seem to be more sensitive to the organism than adults. Pauckova & Fukalova (1968) reported isolating *P. shigelloides* more often from women. Additional, particular surveys on the infection phenomena in children, infants and women are needed.

Of 302 cultures isolated from dogs, cats, fresh water fish, river water and healthy carriers, 196 (64·9%) were typed to 50 serovars and no correlation was observed between serovars and sources. In addition, of 78 isolates which were determined as only H antigens, 13 (16·7%) were typed to 3 serovars, by 2 O antisera of 5 antisera prepared against 5 isolates which were selected at random from the 78 strains mentioned above. As many of the present isolates were of the same serovars as isolates from clinical materials (Shimada & Sakazaki, 1978), the possibility of an epidemiological background suggests itself. However, before any definite conclusions are drawn, the relationship between serovars and pathogenicity must be elucidated.

The pathogenesis of *P. shigelloides* in humans has not been defined to date (Sakazaki *et al.* 1959; Sanyal, Singh & Sen, 1975). However, the organism's pathogenicity is obvious from the case reports of epidemic and sporadic outbreaks. The sources of infection have scarcely been discovered and the distribution of the organism in the environment has not been studied. The present ecological study aids in defining the relationship between epidemic outbreaks and the origin of the organisms.

REFERENCES

COOPER, R. G. & BROWN, G. W. (1968). *Plesiomonas shigelloides* in South Australia. *Journal of Clinical Pathology* **21**, 715–18.

EWING, W. H., HUGH, R. & JOHNSON, J. G. (1961). Studies on the Aeromonas Group. Communicable Disease Center, Atlanta, Georgia, United States, Public Health Service.

FERGUSON, W. W. & HENDERSON, N. D. (1947). Description of strain C27: A motile organism with the major antigen of *Shigella sonnei* phase I. *Journal of Bacteriology* **54**, 179–81.

GEIZER, E., KOPECKY, K. & ALDOVA, E. (1966). Isolation of *Aeromonas shigelloides* in a child. *Journal of Hygiene, Epidemiology, Microbiology and Immunology* **10**, 190–4.

HABS, H. & SCHUBERT, R. H. W. (1962). Über die biochemischen Merkmale und die taxonomische Stellung von *Pseudomonas shigelloides* (Bader). *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, Abt. 1 Originale, **186**, 316–27.

HORI, M., HAYASHI, K., MAESHIMA, K., KIGAWA, M., MIYASATO, T., YONEDA, Y. & HAGIHARA Y. (1966). Food poisoning caused by *Aeromonas shigelloides* with an antigen common to *Shigella dysenteriae 7*. *Journal of Japanese Association of Infectious Disease* **39**, 433–41.

McCarthy, D. H. & Rawle, C. T. (1975). The rapid serological diagnosis of fish furunculosis caused by 'smooth' and 'rough' strains of *Aeromonas salmonicida*. *The Journal of General Microbiology* **86**, 185–7.

Pauckova, V. & Fukalova, A. (1968). Occurrence of *Aeromonas hydrophila* and *Aeromonas shigelloides* in faeces. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, Abt. 1 Originale, **206**, 212–16.

Sakazaki, R., Funaoka, S., Nakaya, R. & Fukumi, H. (1959). Studies on so-called Paracolon C27 (Furguson). *Japanese Journal of Medical Science and Biology* **12**, 355–63.

Sakazaki, R., Tamura, K., Prescott, L. M., Bencic, Z., Sanyal, S. C. & Sinha, R. (1971). Bacteriological examination of diarrheal stools in Calcutta. *The Indian Journal of Medical Research* **59**, 1025–34.

Sanyal, S. C., Singh, S. J. & Sen, P. C. (1975). Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *The Journal of Medical Microbiology* **8**, 195–8.

Schmid, E. E., Velaudapillai, T. & Niles, G. R. (1954). Study of Paracolon organisms with the major antigen of *Shigella sonnei*, form 1. *Journal of Bacteriology* **68**, 50–2.

Shimada, T. & Sakazaki, R. (1978). On the serology of *Plesiomonas shigelloides*. *Japanese Journal of Medical Science and Biology* **31**, 135–42.

Tsukamoto, T., Kinoshita, Y., Shimada, T. & Sakazaki, R. (1978). Two epidemics of diarrhoeal disease possibly caused by *Plesiomonas shigelloides*. *Journal of Hygiene* **80**, 275–80.

Ueda, S., Yamasaki, S. & Hori, M. (1963). The isolation of Paracolon C27 and halophilic organisms from an outbreak of food poisoning. *Japanese Journal of Public Health* **10**, 67–70.