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'VIBRIO PARAHEMOLYTICUS' RELATED
GASTROENTERITIS IN DJAKARTA, INDONESIA

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**Vibrio Parahemolyticus Related Gastroenteritis
in Djakarta, Indonesia †**

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Vibrio parahemolyticus has been isolated and reported from Japan, Korea, Shanghai, China, Singapore, Ceylon, Hawaii, Hong Kong, India, Taiwan and Thailand (Fifield, 1971). In Japan, this organism has been associated with a gastroenteritis chiefly characterized by diarrhea, abdominal pain, vomiting and mild fever. The seasonal occurrence of this illness, commonly known as "Summer diarrhea", is evidenced by frequent food poisoning outbreaks from June to October (Zen-Yoji *et al.*, 1965).

Vibrio parahemolyticus was first implicated in 1951 in an outbreak of food poisoning in Japan after isolation from dried salted sardines (Fujino *et al.*, 1951). Since then other seafoods, rice and cooking utensils have also revealed the presence of this vibrio. Its ubiquitous nature in other Southeast Asian Countries has led us to determine its presence in Indonesia in association with diarrheal disease.

MATERIALS AND METHODS

One thousand rectal or stool swabs were collected from 467 individuals in Djakarta with diarrheal disease by hospital or municipal health personnel from February to July 1971. Several areas of Djakarta were represented.

The swabs were placed in Cary-Blair and alkaline peptone broth immediately after collection. Cary-Blair swabs were transferred to and incubated in alkaline peptone broth for 8 hours at 37°C prior to sub-culture on TCBS agar. Original alkaline-peptone swabs were incubated for 8 hours and then sub-cultured to TCBS agar. In both instances, second passages were made to alkaline peptone broth and again to TCBS after 8 hours incubation before finally determining the specimen to be negative. Determinations for other enteric pathogens were made in the usual manner.

Colonies typical of *V. parahemolyticus* were isolated and characterized biochemically as shown in Table I. Unless indicated otherwise, all media listed were modified to contain 2.0% NaCl (Sakazaki 1965). Serological characterization was performed according to the method of Sakazaki, Iwanami and Tamura, determining O and K antigenic structure (Sakazaki *et al.*, 1968) (Table II).

The isolated strains were tested for antibiotic susceptibility according to the method of Kirby and Bauer, using the following antibiotics: Ampicillin (10 mcg), Cephalothin (30 mcg), Chloromycetin (30 mcg), Gantrisin (300 mcg), Kanamycin (30 mcg), Streptomycin (10 mcg), Tetracycline (30 mcg), and Polymyxin B (300 units) as shown in Table III.

RESULTS

From a total of 1,000 specimens submitted from 457 individuals, 48 positive strains were isolated. The number of individuals found to harbor *V. parahemolyticus* was 19 (Table IV). Generally samples collected ranged from one to several per individual. No other enteric pathogens were isolated from these individuals. Biochemical reactions: (Table 1)

All strains tested were oxidase, catalase and motility positive; produced acid from glucose, mannitol and maltose in CTA medium but not from lactose, sucrose or xylose. Nitrates were reduced and growth obtained in MR-VP media with 2% NaCl added and in lysine decarboxylase medium with 2% NaCl added. Determinations

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for urease activity and H₂S production after 24 hours incubation were negative. Reaction in Kligler's iron agar was alkaline over acid.

TABLE I
Biochemical and Physiological
Characterization

Character	Reaction
Acid Production:	
Glucose	+
Lactose	—
Sucrose	—
Mannitol	+
Xylose	—
Maltose	+
Catalase	+
Urease	—
Nitrate Reduced	+
Growth in MR-VP medium:	
without 2% NaCl	NG
with 2% NaCl	G
Hemolysis:	
Sheep RBC	+
Human RBC	+
Citrate utilization	+
Growth in Lysine dicarboxylase medium:	
without 2% NaCl	NG
with 2% NaCl	G
*H ₂ S produced	—
Kligler's Iron Agar	K/A
Oxidase reaction	+
Motility	+

* Pos. in KIA after 72 hr at R_o.

All of the isolated strains were hemolytic on sheep and human blood agar. Morphologically, the cultures were found to be smooth and/or rough with smooth predominating.

Serology: (Table II)

Serotyping of 15 strains revealed 4 to be K-38; 3 were K-3; 1 each to be K-7, K-17, K-19 and K-32 respectively, while 4 were 0-1 with the K antigen non-typable.

Drug susceptibility: (Table III)

Chloromycetin and tetracycline appeared to present the most obvious effective *invitro* activity in restricting growth, while Kanamycin, Streptomycin and Polymyxin B were partially effective. Ampicillin, Cephalothin and Gantrisin were unremarkable.

Geographical distribution: (Table IV)

Isolations represented all areas of Djakarta with Central and South Djakarta predominating. There were five isolates for which the area was unknown or were from individuals in closely outlying districts.

TABLE II
Serological Classification

Serotype	%
K - 3	3/15 (20 %)
K - 8	1/15 (6.6%)
K - 17	1/15 (6.6%)
K - 19	1/15 (6.6%)
K - 32	1/15 (6.6%)
K - 38	4/15 (26.6%)
O-1, K non-typable	4/15 (26.6%)

TABLE III
Drug Susceptibility of 24 Strains Examined

Agent	* Reaction	Frequency
Ampicillin	R	1.00
Cephalothin:	R	.835
	I	.165
Chloromycetin	S	1.00
Gantrisin	R	1.00
Kanamycin	I	1.00
Streptomycin:	S	.083
	I	.792
	R	.125
Tetracycline:	S	.835
	I	.165
Polymyxin B:	S	.167
	I	.792
	R	.041

* S=Sensitive; I=Partial; R=Resistant

TABLE IV
Geographical Distribution of Positive Cases

Area	# of Cases
Central Djakarta	6
North Djakarta	2
South Djakarta	4
East Djakarta	1
West Djakarta	1
Other	5
Total:	<u>19</u>

DISCUSSION

The large number of isolates obtained in this preliminary study has obviously provided the primary information sought; namely, the occurrence of *V. parahemolyticus* in Indonesia. This once again presents an instance where an organism is not so rarely found as rarely identified. The presence of *V. parahemolyticus* in individuals suffering from acute gastroenteritis strongly implicates this organism as a heretofore unknown aetiologic agent.

Though the organism has not been found in large numbers nor associated with endemic or epidemic occurrences, sufficient numbers of strains have been isolated to confirm it as a possible significant cause of diarrheal disease. The role of *V. parahemolyticus* in large outbreaks of gastro-intestinal disease in Indonesia remains to be confirmed and amplified.

The significance of rough colonies is presently under study to determine possible differences in pathogenicity between the rough and smooth strains. Additionally, the non-typable strains are being serologically characterized to determine if they represent previously un-isolated strains of *V. parahemolyticus*.

Thus far, it is difficult to determine seasonal incidences of this illness or associate it with particular geographical areas. This study has been expanded to cover other areas of the vast Indonesian archipelago to provide information of this nature.

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