Vibrio Parahaemolyticus Gastroenteritis in Malaysia

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Introduction

DIARRHOEAL DISEASES are a problem all over the world especially in tropical and sub-tropical countries. The more common pathogens such as the **Salmonellae,** including the Arizona serotypes, **Shigellae,** Enteropathogenic **Escherichia coli** and certain parasites are often isolated from such infections. But in more than half the cases presenting with diarrhoea no known pathogens can be isolated. Virological studies on stool specimens from diarrhoeal cases are increasingly being asked for but the correlation between viral isolates and clinical symptoms have been disappointing.

Some of the lesser known and studied organisms have been identified as aetiological agents of diarrhoeal diseases. Some of these are **Edwardsiella tarda** belonging to the family Enterobacteriaceae (Ewing et al., 1965; Bhat Prema et al., 1967) and **Plesiomonas shigelloides** (Ampalam and Fang, 1971) an organism closely resembling the **Aeromonas** and **Shigella** organisms, which have been implicated in infantile diarrhoea.

Another organism which is emerging as a significant cause of gastroenteritis is **Vibrio parahaemolyticus,** a halophilic, gram negative rod primarily of marine origin.

This organism was first described in 1951 by Fujino (Fujino et al., 1953) in Japan as the aetiological agent of "summer diarrhoea". In one outbreak it was shown that this organism was the aetiological agent for 60-70% of the cases (Smith, 1971). The original outbreak of food-poisoning was associated with the eating of dried salted sardines (Fujino et al., 1953), but later it was also found to be associated with the consumption of raw fish known as "sushi".

Since the discovery in Japan that Vibrio parahaemolyticus is an organism capable of giving rise to food-poisoning or gastroenteritis type of infections, many other countries have isolated and implicated this organism as the cause of diarrhoeal diseases e.g. Ceylon, Hawaii, Hong Kong, Taiwan, Phillipines, Thailand, Korea and China (Fifield, 1971).

The present study was inspired by the fact that the Chinese in this country eat raw pickled fish after the Chinese New Year and that this may give rise to food-poisoning outbreaks, but later it was learnt that the particular fish eaten after the Chinese New Year is a fresh water fish. Nevertheless, once we started looking for **Vibrio parahaemolyticus,** there was no difficulty in isolating the organism from cases of gastroenteritis.

Materials and Methods

Stool samples from patients with gastroenteritis were received by the Department of Medical Microbiology, Faculty of Medicine. These specimens were from both in-patients as well as from out-patients of the University Hospital. The specimens were cultured on both solid and enrichement media for the usual intestinal pathogens such as **Salmonella, Shigella** and Enteropathogenic **Escherichia coli.** In addition, the specimens were cultured on media selective for **Vibrio parahaemolyticus.** These were thiosulphate citrate bile salts sucrose

agar — TCBS (Oxoid) and alkaline peptone water followed by subculture onto TCBS agar. **Vibrio parahaemolyticus** was usually isolated as a pure and heavy growth on the direct TCBS plate.

Bacteriological Features

Vibrio parahaemolyticus is a gram negative motile rod. After overnight incubation, the organism appears on TCBS medium as large (2-4 mm), smooth, green colonies, with no discolouration of the medium. They are oxidase positive and catalase positive on subculture and sucrose negative. This is in contrast to Vibrio cholerae colonies which are sucrose fermenting and hence appear on TCBS medium as bright yellow colonies with yellow discolouration of the surrounding medium.

Suspensions of Vibrio parahaemolyticus organisms do not agglutinate with the polyvalent Vibrio cholerae antiserum.

Table 1 gives the bacteriological features of the organism. All testswere performed at 37°C with media containing 1-2% sodium chloride. Serotyping and the Kanagawa test for potential pathogenicity was done on all the isolates.

Table 1

Hugh & Leifson's Test	Fermentative
Motility	+
Oxidase	+
Catalase	+
Glucose (gas)	—
Glucose (acid)	+
Lactose (acid)	—
Mannitol (acid)	+
Sucrose (acid)	—
Dulcitol (acid)	—
Gelatin liquefaction	+
Hydrogen sulphide production	—
Methyl red test	+
Voges-Proskauer reaction	—
Indole production	+
Urease	—
Arginine decarboxylase	—
Lysine decarboxylase	+
Orinithine decarboxylase	+

Results

A total of 7 strains were isolated throughout 1972 and these belonged to 5 different serotypes. The Kanagawa phenomenon was positive for all except one strain. Serotyping of the strains was done by Dr. G. I. Barrow of Turo, Cornwall, England. Table II gives the serotypes of the isolates.

Discussion

Vibrio parahaemolyticus was isolated from 4 out-patients and 3 in-patients, whose ages ranged from 15 years to 40 years. Two were females and 5 were males.

Table II

Strain	O antigen	K antigen	Kanagawa test
1	014	K42	—
2	07	K19	+
3	03	K7	+
4	03	K7	+
5	03	K29	+
6	03	K29	+
7	04	K9	+

The main presenting symptoms were diarrhoea and vomiting which was present in all the patients. The frequency of diarrhoea had a wide range from 3 to 4 times a day to 15 times a day. Vomiting was severe in only 2 patients. Abdominal pain was present in 4. Fever was notably absent in all the 7 patients. Two patients had mucus in the stools and only one had bloody diarrhoea. No "rice-water" stools were observed in any of the patients.

In August 1971, there was an outbreak of gastroenteritis in 320 of 550 persons attending a picnic in Maryland, U.S.A. Their symptoms included diarrhoea (98%), severe abdominal cramps (78%), nausea (76%), vomiting (74%), fever (26%), headache (25%) and chills (10%) (Wkly. epidem. Rec. 1971). These findings differ slightly from ours in that none of our patients had fever, nausea, headache or chills. This might be due to smaller numbers of the organisms ingested or some other factors such as antigenic differences in the organisms resulting in variation in the virulence.

The disease itself was self-limiting and did not last more than 48 hours. This correlates well with the Maryland outbreak where the mean duration of illness was 2 days (range 1-5 days).

Three of our patients were treated symptomatically with kaolin, 3 were given tetracyclines and one sulphathalazole. All follow up stool cultures were negative for Vibrio parahaemolyticus. If patients are treated with antibiotics they practically cease to excrete the organism after the 5th day with an occasional positive being observed now and then (Kasai, 1971).

All strains of Vibrio parahaemolyticus have identical H antigens. Most of the strains isolated from human sources can be typed using 11 specific 0 antisera and 52 K antisera. Although an 0 type can have more than one K type, individual K types occur in only one 0 group. There were no predominant serotypes although 2 had 03/K7 antigens and another 2 strains had 03/K29 antigens.

The "Kanagawa phenomenon" (Miyamoto et al., 1969) is a test for the potential pathogenicity of a strain and is based on the ability of the organism to produce haemolysis on fresh human blood-agar media. Non-haemolytic strains are said to be non-pathogenic but if ingested in large quantities can cause disease. Only one strain out of our 7 was Kanagawa negative but this patient had very severe diarrhoea, about 15 times a day, and no other intestinal pathogens were isolated from his stools.

The "Kanagawa phenomenon" is thus not a very clear indication of pathogenicity. It is hoped that an "enteropathogenic factor" such as an exotoxin or endotoxin, if demonstrable, might be a better indication of pathogenicity or specific serotypes as in Enteropathogenic Escherichia coli.

In volunteer experiments it was observed that 6-8 hours incubation was required for the haemolytic variant to cause the initiation of the disease, whereas the non-haemolytic variant required approximately 18 hours (Kasai, 1971) and the infecting dose was seen to be approximately 10^6 organisms.

It is difficult to ascertain the incubation period in the 7 patients for although in Japan and in other places Vibrio parahaemolyticus gastroenteritis is associated with eating of raw fish or other sea food, none of our 7 patients gave a history of having eaten raw fish. Three of our patients were Indian and one was a Malay and both races under normal circumtances never eat raw fish or even lightly cooked sea food. The rest of the patients were Chinese and they too denied having taken raw fish or sea-food, prior to the onset of symptoms.

Although in Japan gastroenteritis due to Vibrio parahaemolyticus is associated with eating raw fish, in other parts of the world Vibrio parahaemolyticus has been isolated from cases of gastroenteritis after eating sea-food that had been inadequately cooked (Peffers, 1973).

In India Vibrio parahaemolyticus gastroenteritis is not associated with the consumption of raw fish (Zakazaki et al., 1971). It is possible that in Malaya, food, not necessarily sea-food, gets contaminated with this organism which is of marine origin. Since the generation time of the organism is very short (at 37°C it is 12-15 min.) few organisms can multiply quickly to give adequate numbers to produce the pathogenic effect especially if the food is not kept refrigerated.

It is obvious that Vibrio parahaemolyticus is one of the causes of food-poisoning in Malaya and hence it is important that selective methods for its isolation are included when stool samples are cultured for the usual intestinal and food-poisoning pathogens. Vibrio cholerae (i.e. Classical or el Tor) if present can also be isolated on the same media as Vibrio parahaemolyticus.

Nothing is known about the epidemiology of Vibrio parahaemolyticus in Malaysia. So far we we have not examined any sea-food for its presence but we hope to do so in the near future.

Summary

7 strains of Vibrio parahaemolyticus were siolated throughout 1972 from patients with gastroenteritis. Predominant symptoms were diarrhoea and vomiting with the mean duration of illness being 24-48 hours. Methods of isolation and identification of the organisms are described and the importance of looking for Vibrio parahaemolyticus stressed.

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