

Outbreaks of Food-Poisoning due to *Vibrio parahaemolyticus* among Japanese Touring Groups Returning from Hong Kong

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Abstract

In October 1968, outbreaks of acute gastroenteritis occurred among five Japanese sightseeing groups during their stay in Hong Kong and/or on their homeward return flight. Bacteriologic examination of fecal specimens obtained from 62 of 144 tour members of the third group led to the isolation of 14 *Vibrio parahaemolyticus* strains with hemolytic activity. No enteric pathogens were isolated from three patients of the fifth party who also had symptoms. As ill persons were rare in the other groups and those who had diarrhea already recovered at the time of the inspection, no fecal examination was performed on them. The history of food consumption suggested that raw sea food served in restaurants in Hong Kong may have been the vehicle of infective agent.

When 5 groups of tourists, comprising 591 persons from Tokyo, Shizuoka, Aichi, Hokkai-Do, and other localities in Japan, stayed in Hong Kong and Macao

for 3 days, 78 were taken ill with symptoms of acute gastroenteritis. Most of the sick recovered during their sojourn in that area but some of them who re-

Special Contribution

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Table 1. Gastroenteritis in tour groups returning Tokyo from Hong Kong

| Party | Arrival hour and date (Oct., 1968) | Air Liner* | No. of passengers | No. of patients | No. of examined persons | Status at the time of examination |
|-------|------------------------------------|------------|-------------------|-----------------|-------------------------|-----------------------------------|
| 1 | 08:30, 8th | A | 104 | 2 | 0 | all recovered |
| 2 | 19:40, 9th | B | 142 | 1 | 0 | ditto |
| 3 | 23:00, 9th | C | 144 | 62 | 62 | slight symptoms** |
| 4 | 17:20, 10th | A | 97 | 10 | 0 | all recovered |
| 5 | 08:20, 11th | A | 104 | 3 | 3 | slight symptoms |

*Names withheld. **including "recovered".

turned to Haneda Airport in Tokyo on October 9th and 11th had slight symptoms of gastroenteritis. All returned to Tokyo at times and with symptoms shown in Table 1.

Though Hong Kong was not declared at that time an epidemic cholera area, it has been invaded by El Tor cholera several times since 1962. Furthermore, it was reported by reliable sources that El Tor vibrio was repeatedly detected in night soil collected on the outskirts of Hong Kong. Therefore, 65 of the 591 Japanese tourists who have had or were presented with gastrointestinal symptoms on return were subjected to fecal examination as a precaution at the Tokyo Airport Quarantine Station at Haneda to eliminate the suspicion of cholera. Simultaneously, in obedience of the local municipal law, examination was mandatory for the assumption that shigellosis or food-poisoning due to *Vibrio parahaemolyticus* may be involved.

Methods of Investigation

The passengers, while staying in Hong Kong, ate seafood (mainly lobsters) in restaurants built over water and in similar establishments at Aberdeen, and 78

of them had an attack of diarrhea characterized by 4 to 10 watery stools and vomiting several times a day, and abdominal pain. The body temperature varied between 38°C for one to two days. The seriously ill were treated in Hong Kong. By the time of arrival at Haneda Airport, all passengers of the first, second and fourth parties recovered. Therefore, no fecal examination was performed of them. In the third group, however, the number of patients comprised nearly one-half of all travelers, and there were some persons developing slight symptoms of gastroenteritis at the time of the inspection on arrival. Therefore, stool examination was performed on those who were attacked by the disease. In addition, three passengers of the fifth party were also examined as they still had slight symptoms after landing in Japan.

Spontaneously evacuated fecal specimens were obtained or rectal swabs were collected. The specimens were streaked directly to S. S. agar and inoculated into alkaline peptone water (APW) with 1% NaCl for the enrichment of vibrios. The APW was incubated overnight, then

Table 2. Biochemical characteristics of the isolated strains

| Characteristic | Immediately after isolation | 16 months later | Reference strains | | | | |
|----------------------------|-----------------------------|-----------------|-------------------|------|-----|-----|-----|
| | | | N4 | 102 | 153 | 154 | 156 |
| Growth in 0% NaCl | 14(-)* | 14(-) | - | - | - | - | - |
| 3% NaCl | 14(+) | 14(+) | + | + | + | + | + |
| 7% NaCl | 14(+) | 14(+) | + | + | + | + | + |
| 10% NaCl | 14(-) | 14(±) | - | - | ± | ± | ± |
| Indole test | 14(+) | 14(+) | + | + | + | + | + |
| Voges-Proskauer test | 14(-) | 14(-) | - | - | - | - | - |
| Methyl-red test | 12(-) 2(+) | 14(+) | + | - | + | + | + |
| Hydrogen sulfide formation | 14(-) | 14(-) | - | - | - | - | - |
| Nitrate to nitrite | | 14(+) | + | + | + | + | + |
| Gelatin liquefaction | | 14(+) | + | + | + | + | + |
| Oxidase | 14(+) | 14(+) | + | + | + | + | + |
| Lysine decarboxylase | 14(+) | 14(+) | + | + | + | + | + |
| Arginine dehydrase | | 14(-) | - | + | - | - | - |
| Hugh-Leifson test | | 14(F) | F | F | F | F | F |
| Glucose | 14(+) | 14(+) | + | + | + | + | + |
| Gas from glucose | 14(-) | 14(-) | - | - | - | - | - |
| Arabinose | 12(+) | 2(-) | 11(+) | 3(-) | - | ± | - |
| Cellobiose | | | 10(-) | 4(±) | - | - | - |
| Lactose | 14(-) | 14(-) | - | - | - | - | - |
| Rhamnose | | 14(-) | - | - | - | - | - |
| Sucrose | 14(-) | 14(-) | - | - | - | - | - |
| Dulcitol | | 14(-) | - | - | - | - | - |
| Sorbitol | | 13(-) | 1(+) | - | + | - | - |
| Inositol | | 14(-) | - | - | - | - | - |
| Kanagawa phenomenon | | 13(+) | 1(±) | + | + | + | + |

*No. of strains. Results of tests in ().

streaked to T.C.B.S. and alkaline agar plates. The isolated colonies were further studied (Table 2).

Results

Seventeen strains suspected of being vibrios were cultured but only from patients in the third group of tourists, and 14 of these were identified as *V. parahaemolyticus* by biological and serological tests that gave the following results:

The strains failed to grow on S.S. agar but formed large colonies on T. C. B. S. and alkaline agar plates. The cultures consisted of gram-negative rods with a

single polar flagellum. The organisms were actively motile in S. I. M. medium containing 3% NaCl. The biochemical characteristics of these organisms were determined twice, after isolation and again after 16 months. Furthermore, at the second investigation, they were compared with five reference strains, namely, two *V. parahaemolyticus* isolated from human sources (Takigawa-N4 and Fujino-EB102) and three other strains cultured from marine mud in the port of Singapore in 1964 by Yasunaga (1964.) The results of these studies are

shown in Table 2. Using as parameters the most important characteristics observed immediately after isolation, all 14 strains were identified as *V. parahaemolyticus*. Serologic studies at the same time revealed that they belonged to the following serotypes: 4 strains to K 4, 2 strains to K 14, and one each to K type 3, 10, 15, 17, 19, 33, 38, and 47, respectively. The identification of the strains as well as the results of the K typing were confirmed at the second examination when additional biochemical characteristics were also studied. The strains had been preserved for 16 months by successive transplantations in a medium composed of 1% Tryptone, 1% NaCl, and 0.2% agar. It is worthy mentioning that at the second examination all strains exhibited hemolysis on Wagatsuma's medium, the so-called positive Kanagawa phenomenon which is said to be closely related to human pathogenicity (Miyamoto et al., 1969).

Except for their feeble growth in the presence of 10% NaCl in the medium, the appearance of a positive methyl-red test in all cultures, and the change in the ability to ferment cellobiose within 24 hours by one strain, the organisms showed characteristics that were in close agreement with the results obtained during the first examination. Among the deviations from the usually observed pattern, only the weak growth in 10% NaCl medium seemed to raise questions, but the growth was so insignificant that the definition "scarcely possible to grow" as a taxonomic criterion for the identification of *V. parahaemolyticus* appears applicable also in this instance. Varia-

bility was observed also in the reference strains. It seems that all discrepancies observed did not tip the balance in disfavor of the identification of the strains as *V. parahaemolyticus*.

Discussion

Foodpoisoning caused by *V. parahaemolyticus* had been considered about 10 years a disease particular to Japan. In recent years, more attention has been paid to the existence of this organism in sea water and mud, particularly in estuarine as well as in raw marine products also in other countries, and the possibility of the occurrence of disease of this type among foreigners had been considered. At present, it appears that both problems are more fully realized with the reservation that in the plurality of the published studies the hemolysis test has not been carried out by the recently developed technique called the "Kanagawa phenomenon".

We have had the following information attesting to the occurrence of *V. parahaemolyticus* disease outside Japan:

(a) The prevalence of the disease in Shanghai around 1960 was observed (Ma et al., 1962; Chang et al., 1962).

(b) The isolation of one strain of this organism from a healthy person returning from Pusan, Korea in August 1963 (Oshiro, 1964).

(c) In South Korea, outbreaks of diarrheal diseases caused by *V. parahaemolyticus* and the like organisms have been reported since 1964 (Kee et al., 1967; Lee et al., 1968, 1969; Chun et al., 1970).

(d) The discovery of two *V. parahaemolyticus* strains among 129 NAG (nonagglutinable) vibrios isolated from

patients with diarrhea or from their contacts during field studies of cholera in the Philippines (Sakazaki et al., 1967).

(e) The disease occurred among Japanese ship crew members after eating raw sea food in Pusan, Korea (Abe et al., 1968).

f) In 1969, diarrhoea caused by *V. parahaemolyticus* was first recognized in Calcutta, India (Chatterjee et al., 1970).

(g) In an Australian tropical island resort, in epidemic form, that lasted from December, 1968 to June, 1969 and was caused by a marine vibrio related to *V. parahaemolyticus* (Battey et al., 1970).

(h) In May, July and August 1969, outbreaks of acute gastroenteritis occurred in three separate groups of sightseeing tourists of Americans visiting in Asia, Bangkok, Hong Kong and Tokyo, who became ill on their return flight or after arrival at home in the United States (U. S. National Communicable Disease Center 1969). The last instance strongly resembles the occurrence of the disease described in the present report. In the case of the American tourists, however, three types of enteric pathogens and non-cholera vibrios were isolated from the specimens collected from three groups, among which *V. parahaemolyticus* and non-cholera vibrio were common in all groups.

There is another publication (Twedt et al., 1969) reporting *V. parahaemolyticus* isolated from localized tissue infections in the United States, but it has been stated that no strains have been isolated from cases of food poisoning and other diarrheal diseases there except from travelers to Asia as reported previously.

It is evident from these data that even foreign people who do not like to eat fish or shell fish raw as much as the Japanese, will be attacked occasionally by *V. parahaemolyticus* causing gastroenteritis. Why has the disease of this type occurred only in Asia? In connection with that question, studies on the distribution of this organism, especially of strains showing a positive "Kanagawa phenomenon" in the oceans of Asia and of their marine products, are acquiring an increasing importance. It has been reported by investigators from the Nagasaki University (Yasunaga, 1964, 1965; Takahira, 1965; Harada, 1967; Aoki et al., 1967; Saito, 1970; Horikawa, 1970; Tsutsumi, 1970) that this organism possessing the characteristic hemolytic activity could be detected in muddy sea water or in sediment in ports of Southeast Asia. Three strains of Nos. 153, 154 and 156 respectively used for reference in the present studies were acquired as part of the strains of non-human origin collected during joint investigations of such sources.

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香港帰来の日本人観光団に発生した腸炎ビブリオ食中毒

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摘 要

昭和43年10月、日本人観光団5団体に、香港に滞在中あるいは引きつづき航空機で帰国中に急性胃腸炎が発生した。東京空港検疫所で帰着時まだ軽微な症状を示していた第3群144名中の62名について検便を行ない、うち14名から神奈川現象陽性腸炎ビブリオを検出した。第5群にも3名の有症者がいたがこれからは病原細菌を検出し得なかった。原因食は香港の水上レストランなどで摂った生の海産物と推定された。この事例の考察にあたり、神奈川現象陽性の本菌が東南アジアの港湾泥土から検出されたこと、および日本以外の国々での本菌ないしその類似菌による食中毒発生例が文献によって概観された。