SOURCE OF VIBRIO PARAHAEMOLYTICUS INFECTION

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INTRODUCTION

The incidence of acute gastroenteritis in the community varies with the eating habit of the population. In Singapore where a high percentage of the population takes to eating at least one meal a day from the hawkers, the spread of any food-borne disease can be extremely rapid. Conversely, the spread of any food-borne disease in Singapore can be quickly halted with the strict adherence to personal hygiene and the avoidance of uncooked and cold food as was shown by the handling of the 1972 Cholera Epidemic in Singapore.

During the course of study on the bacterial causes of gastroenteritis in general practice in Singapore, we isolated *Vibrio parahaemolyticus* from 3 patients who presented with acute gastroenteritis. This organism was first described by Fujino (1953) as a cause of food poisoning. Subsequently many isolations have been found in various fish and shellfish such as the haddock (Kampelmacher *et al*, 1970), crab (Krantz *et al*, 1969), oyster (Baross and Liston, 1970) and shrimp (Centre for Disease Control, 1973).

In Singapore, the organism was not isolated from patients until 9th September, 1973 when the *Vibrio parahaemolyticus* was first isolated from a young male student. Subsequent to that case, a few more isolations of *Vibrio parahaemolyticus* from gastroenteritis patients have been made by Lam *et al.*

In this report, we have been able to obtain fairly detailed histories of food consumption prior to the onset of illness from three patients from whom *Vibrio parahaemolyticus* was isolated. Two of the patients gave a distinct history of taking 'see-hum'—(cockles)—a local variety of shellfish (mollusc anadaria granosa (linnaeus)), a few hours before the first attack of diarrhoeae which was the presenting symptom.

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MATERIALS AND METHODS

Watery stools from patients with gastroenteritis (or rectal swabs in Cary-Blair medium) were inoculated into the following media:

E.M.B. (Eosin methylene blue)

S-S (Salmonella-Shigella medium)

T.C.B.S. (Thiosulphate citrate bile-salt sucrose)

Selenite broth

1% peptone water (with 3% NaCl)

After 37°C incubation overnight, the broth cultures from the enrichment media were plated on D.C.A. (from Selenite broth) and T.C.B.S. (from peptone-salt medium). Cultures were negative for Salmonella and Shigella organisms. The halophilic bacteria appeared as green mucoid colonies (non-sucrose fermenters) on the T.C.B.S. The biochemical reactions carried out conformed to those described by Sakazaki ("*V. parahaemolyticus* —Isolation and Identification" Eiken). Following the positive isolates from stool cultures, specimens of 'see-hum' or cockles suspected to be the common causative agent of the infections, were obtained and cultured for *V. parahaemolyticus*. The cockles were finely cut up and inoculated into T.C.B.S. and 1% peptone water (with 3% NaCl) and incubated at 37°C, overnight. The *V. parahaemolyticus* isolated was sensitive to tetracycline, chloramphenicol, Septrin, Neomycin, Sulphonamide, and Ampicillin. All the strains (including that from the cockles) were haemolytic on Wagatsuma medium i.e. positive for Kanagawa phenomenon), although it has been reported that about 96% of the strains from patients are Kanagawa positive and 99% from marine sources are Kanagawa negative (Sakazaki *et al*, 1968). The identification of our strains were confirmed by the Colorado State Public Health Laboratory and the Centre for Disease Control, Atlanta.

Case 1

N.S.B. a 42 year old Chinese male fitter presented on the 4/11/73 with a history of passing watery motions over the past 24 hours.

On 3/11/73, his wife purchased 2 katis of cockles from the market and on returning home in the evening, he had it for dinner.* His wife

* The traditional way of preparing the cockles is to pour boiling water over the shellfish or to dip it into a pot of boiling water for a few seconds by which means the shellfish will "open". The cockles are consumed half cooked, usually with some chilli sauce.

had the cockles as well on the 3/11/73 evening but had some left over which were kept in the refrigerator. Next morning, his wife, prepared some noodle soup and added the remaining cockles to the preparation. N.S.B. had the cockles-noodles soup and he went to work. At 1 p.m., he had the first attack of watery motion with no blood or mucus. In all he had 5 attacks of watery motions over the next 18 hours. He felt tired but did not have abdominal pain, chills, headache or vomiting. Clinically he was afebrile, with no localised tenderness over the abdomen. A rectal swab was taken and sent for culture from which *vibrio parahaemolyticus* was isolated.

He was given kaolin with morphine mixture and antispasmotic tablets. He had two further attacks of watery motions and recovered the following day. His wife did not get the gastroenteritis. A stool specimen was sent for repeat culture on 19/11/73. It was found to be negative for pathogenic organisms.

Case 2

S. D., an Indian male, factory worker presented with an acute illness on the 12/11/73.

On the 11/11/73, at 5 p.m. he took some 'rojak' (salad) prepared by an Indian hawker. As far as he could remember, the salad contained various types of vegetables, prawns and cuttlefish. At 6.45 p.m., he attended a dinner party where alcoholic drinks and spicy food were served. On the way home from the party at 12 midnight, he had some mutton gravy with the 'chepati' (Indian bread). He went to bed at 12.30 a.m. but was woken up at 6.30 a.m. by the severe abdominal pain accompanied by vomiting, giddiness and weakness. He passed 5 watery motions (no blood or mucus) before consulting the doctor. Clinically, he looked tired with a mild fever (99°F). There was tenderness over the whole abdomen with increased bowel sounds. A rectal swab was taken and sent for culture from which *vibrio parahaemolyticus* was isolated.

He was given symptomatic treatment with mixture of kaolin with morphine and antispasmotic. He improved with treatment. On the following day he passed two soft motions, generally he felt much better and was able to resume work.

A specimen of stools was sent for repeat culture on 21/11/73 and no pathogenic organisms were isolated.

Case 3

T.G.S., a 24 year old Chinese male, works as a Stock Handler in a factory. He presented on the 12/11/73 with a history of diarrhoea.

On the 11/11/73 morning, he had a noodle soup for breakfast, fried fish, eggs with chilli and rice for lunch; and fried mee-hoon (rice noodle) with 'see-hum' at midnight. He was woken up at 2 a.m. by an attack of abdominal pain with watery motion. Prior to consulting the doctor, he had 5 attacks of watery motions. He had a slight temperature of 99°F. A rectal swab was taken and sent for culture from which *vibrio parahaemolyticus* was isolated.

He was treated symptomatically and recovered. A repeat rectal swab done on 14/11/73 revealed no pathogenic organisms.

CULTURE FROM FOOD

Following the result of the Case 1, one of us ordered a plate of fried "kooi teow" (a flat type of rice noodles) with cockles on 18/11/73. The shellfish was put into 2 containers, 4 specimens in each container. Both these were sent for culture. One of the containers of cockles resulted in a positive culture for *vibrio parahaemolyticus.*

The hawker informed us that most of the shellfish are imported from Malaysia and come in "gunny sacks". He buys the shellfish from the market and he removes the meat from the shells in the afternoon without washing them. The meat of the shellfish is then contained in a bowl. This is then kept in the refrigerator for use the following day.

During the preparation of the fried rice noodle, the bowl of shellfish is kept near the frying pan, keeping it at an ideal temperature for incubation. The meat of the shellfish is best eaten half cooked because over cooking will make the meat very tough.

DISCUSSION

The successful isolation of *V. parahaemolyticus* from cockles strongly implicates this food item in the first and third patients. In the first patient his wife also took the cockles the night before, but did not show any symptoms. The infective dose is in the region of 10^6 organisms (Kasai, 1971). It is probable that the inoculum present was small. However, the next day the organism had multiplied sufficiently in the remaining cockles to cause symptoms in the patient.

The organism is easily killed by mild heat although it can remain viable at low temperatures for long duration. (Johnston and Liston, 1973). It is, however, the common practice amongst the Singaporeans to prepare the cockles half-cooked. Both these cases and the finding of the organism in the cockles from the fried noodles indicate

that the manner of cooking the cockles does not allow sufficient heat to kill the organisms in the cockles.

Although *V. parahaemolyticus* appears to be a fairly common cause of food-poisoning in Singapore, the disease is fortunately usually self-limiting. All the three patients recovered without any anti-microbial agents.

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