

Occurrence of Vibrio parahaemolyticus in Thailand

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OBJECTIVE: Vibrio parahaemolyticus has been reported to be the cause of up to 70% of the "summer" diarrhea in Japan. It has been recovered from sea water, fish, and diarrheal stools in many other countries of SE Asia. V. parahaemolyticus has never been reported from any source in Thailand. This laboratory developed the capability to culture appropriate specimens on specific media. Our first attempts to recover this halophilic organism were met with success as we found it in fish obtained at local markets. Additional specimens from both natural sources and diarrheal specimens were studied to determine the extent of occurrence of this possibly pathogenic organism in Thailand.

DESCRIPTION: Specimens of sea water, sand, and sea fish were obtained from beaches and markets in the area. Diarrheal specimens were submitted from Bamras Infectious Hospital and from US Hospitals in Thailand. Specimens were maintained in alkaline peptone water with 3% NaCl added or were streaked directly into thiosulfate citrate bile salts--sucrose (TCBS) media. Colonies were picked at 24 hours after 37C incubation and identified by biochemical and serological methods. The criteria used for identifying V. parahaemolyticus are given in Table 1.

PROGRESS: Sand specimens collected along several beaches in Thailand have revealed 17 positive cultures of 50 specimens collected. Ten sea water specimens of 25 have had V. parahaemolyticus recovered from them. Sea fish cultures have produced 51 positive for V. parahaemolyticus out of 111 mollusks, 31 positive from 43 crab specimens, 59 positive from 85 fish, and 23 positive of 27 shrimp. Only 1 squid of 16 was found to harbour this halophilic organism. It is interesting to note that 4 or 5 catfish obtained from brackish water harboured V. parahaemolyticus in gills, intestine, and on the skin. These data are presented in Table 2.

Diarrheal specimens from an infectious disease hospital were submitted to this laboratory for confirmation for 6 weeks in November and December 1970. Of approximately 300 diarrheal patients seen during that time, there were 59 isolates of V. parahaemolyticus and only 40 of Salmonella sp. and/or Shigella sp.

From 100 diarrheal specimens from children, only 1 isolate of V. parahaemolyticus was recovered. The same recovery rate of 1% was obtained for 100 non-diarrheal adults. One non-diarrheal specimen obtained from 22 youths (aged 5-15 years) revealed V. parahaemolyticus. We have identified this organism in 6 US personnel suffering from gastroenteritis seen at the US Hospital in Bangkok.

Vibrio parahaemolyticus has recently been reported to be associated with severe skin lesions resulting in gangrene and intravascular coagulation. This laboratory undertook to study these findings. Subcutaneous injections of V. parahaemolyticus into the legs of white mice has resulted in the production of progressive sloughing lesions, paralysis of the limb, and death with septicemia. Additional studies are underway.

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In an effort to relate our data to that reported in the literature by Japanese investigators, growth in 10% NaCl solution and haemolysin production on Wagatsuma blood agar was studied. Japanese data indicates a lack of haemolysin in isolates obtained from sea water and sea fish. Our data indicates that 393 of 450 (85%) isolates from 'natural' sources demonstrated haemolysis on this special media. Isolates from human sources produced haemolysin with 119 of 120 isolates. Lack of growth in alkaline peptone water with 10% NaCl has been used as a criteria for V. parahaemolyticus in the Japanese reports. Unlike their data, our isolates do grow in this medium. No isolates of 113 from human sources failed to grow in this medium, while only 122 of 494 isolates from fish, sea water, etc failed to grow in 10% NaCl.

The halophilic properties of this organism are subject to several factors. In this laboratory we have noticed differences due to amount of original inoculum, aeration of the media, source of the original inoculum, and type of media in which the halophilism is being tested. We find that the organism is tolerant to concentrations of NaCl higher than 15%. Additional definitions of halophilism are being investigated.

SUMMARY: There appears to be some real differences in the V. parahaemolyticus isolated from natural sea sources in Thailand and in Japan. More than one half of the isolates we have recovered are untypable against the sera obtained from Japanese cultures. This may be due to nutrients available to the organism or to water temperature differences. We are studying some possible reasons for these differences.

While we have little epidemiological evidence of pathogenicity, we suspect that this organism may be a significant factor in diarrhea in Thailand. We are currently examining several models of pathogenicity.

This laboratory plans to continue and elaborate upon the work concerned with cutaneous entry of V. parahaemolyticus into susceptible hosts and the definition of such infections.

Table 1.
Characteristics of Vibrio parahaemolyticus and Vibrio alginolyticus

	<u>V. parahaemolyticus</u>	<u>V. alginolyticus</u>
Gram stain	-	-
Growth in alkaline peptone water		
with 0% NaCl	-	-
with 3% NaCl	+	+
with 7% NaCl	+	+
with 10% NaCl	-	+
Urease	-	-
Citrate (Simmon)	+	+
Nitrate	+	+
Motility (semi solid mannitol with 1% NaCl)	+	+
Voges-Proskauer	-	+
Indol	+	+
Methyl Red	+	-
H ₂ S (in TSI with 3% NaCl)	-	-
Glucose	+	+
Lactose	-	-
Maltose	+	+
Mannitol	+	+
Xylose	-	-
Arabinose	+	-
Sucrose	-	+
Oxidase	+	+
TSI with 3% NaCl	acid butt/alkaline slant	

Table 2.

Isolates of Vibrio parahaemolyticus from Natural Sources in Thailand

<u>Source</u>	<u>Specimen examined/Specimen with V. parahaemolyticus</u>
Sand	50/17
Sea water	25/10
Mollusks	111/51
Crab	43/31
Fish	85/59
Shrimp	27/23
Squid	16/1