Transition of Kanagawa Negative Isolate of *Vibrio parahaemolyticus* to Kanagawa Positive

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OBJECTIVE: To study the possible development of a beta haemolysin in a Kanagawa negative strain of *Vibrio parahaemolyticus*.

BACKGROUND: The ability of strains of *V. parahaemolyticus* to haemolyze human or rabbit red blood cells in Wagutsuma agar has been used to signify pathogenicity for humans. ([1]) ([2]) This test is based on the fact that some investigators have noted that isolates of *V. parahaemolyticus* obtained from human diarrhea sources are rather universally haemolytic (Kanagawa positive) while those from natural sources (sea foods, sea water) are usually nonhaemolytic. Our findings in Thailand, however, show that the vast majority of natural isolates from the warmer coastal water of the Gulf of Siam are predominately Kanagawa positive — as are our isolates from human diarrhea. ([3])

While there is no data indicating that the route of human infection is other than the eating of contaminated sea foods; the question that remains is how does apparently nonpathogenic (Kanawa negative) *V. parahaemolyticus* become pathogenic (Kanagawa positive)?

This laboratory obtained a Kanagawa negative isolate (F/674) in December 1972 from Dr. Richard J. Gilbert of the Central Public Health Laboratory in England. This isolate was originally from a natural source in Japan. Having in our laboratory, for the first time, a 100% Kanagawa negative strain of *V. parahaemolyticus*, we designed an experiment to study any possible natural transition from Kanagawa negative to Kanagawa positive in this isolate.

DESCRIPTION: *V. parahaemolyticus*, strain F/674, was grown in brain heart infusion (BHI) broth with 3% NaCl to a density of 10^7 cells/ml. A series of mouse passages was initiated. For each mouse passage, 0.5 ml of this culture was injected into the peritoneal cavity of each of two 21 — 33 gram mice. Within 4 — 5 hours, the animals were clinically ill and often had bloody stools. We were, however, never able to recover *V. parahaemolyticus* from these stools. Within 7 — 8 hours after the intraperitoneal injection, the mice were usually dead. Dead mice were stored at 4°C overnight and aseptically opened for examination the next moring, 24 hours after the initial inoculation.

Opened mice were examined and cultures made from the peritoneal cavity onto thiosulfate — citrate — bile salts (TCBS) agar. Pure cultures of *V. parahaemolyticus*, F/674, were always obtained from these animals. In the instance of obviously severe trauma, possibly resulting in peritoneal contamination from the intestine, the specimen was withheld from the study. A second cotton swab was then used to absorb additional material from the peritoneum. This swab was placed in 2 ml of BHI with 3% NaCl and swirled about in an effort to wash the material from the swab. The *V. parahaemolyticus* from this material was considered as a complete mouse passage. 0.5 ml. of this material was again injected into the peritoneal cavity of mice for another mouse passage. The entire process was repeated. The same culture was also immediately tested for haemolytic activity on Wagutsuma agar.

Table 1 shows the observation of haemolysis on Wagutsuma agar from duplicate mouse passages. Controls included inoculation onto the Wagutsuma agar of a nonhaemolytic *Escherichia coli*, and of a haemolytic isolate of *V. parahaemolyticus*. Each plate was also inoculated with the F/674 strain that had not been passed in the mouse. While the test occasionally appears somewhat insensitive, in every case this isolate of unpassed F/674 was either nonhaemolytic or "±". In no case was this artificially cultured strain (cultivated on thiosulfate — citrate — bile salts (TCBS) agar, brain heart infusion with 3% NaCl, etc.) more haemolytic than the identical strain maintained *in vivo* in the mouse.

DISCUSSION: It has consistently been our experience that variations in the osmotic fragility of the rabbit or human erythrocyte affect the outcome of the haemolysis test. Results of the Kanagawa test in our laboratory are always more varied (usually more sensitive) after the Wagutsuma agar is more than eight days old. In order to alleviate the possibility that our media contributed to the above results, isolates of *Vibrio parahaemolyticus* were sent to other laboratories in the United States. These facilities confirmed our results on unidentified strains of our test organisms. We also feel confident that our media, manufactured each week in our laboratory, give more consistent results than the commercially available media.

Additional work is in progress to define the haemolysin in these *in vivo* passed strains. The work presented here is a preliminary study and only a beginning step in the determination of why human isolates are Kanagawa positive and natural isolates (presumably the source of human infections), Kanagawa negative.

REFERENCES:

1. Sakazaki, R.: The Present Status of Studies on *Vibrio parahaemolyticus* in Japan. Seminar for FDA, Washington, D.C., July 30, 1971.
2. Dadisman, T.A., Jr., et al: *Vibrio parahaemolyticus* gastroenteritis in Maryland: Am. J. Epid., 96 # 6, 1973
3. M'cMinn, M.T., et al: *Vibrio parahaemolyticus* in Thailand. SEATO Annual Report, 1972.

Table 1.

Haemolysis of *Vibrio parahaemolyticus* F/674 after passage in mouse peritoneum

Mouse Passage	F/674		Control	
	Mouse A	Mouse B	1*	2*
0	–	–	–	3+
5	±	±	–	3+
8	±	1+	–	3+
10	1+	±	±	3+
13	2+	4+	–	4+
17	2+	2+	±	3+
21	2+	3+	–	3+
24	3+	3+	±	4+
28	2+	2+	–	3+
30	2+	2+	–	2+
34	3+	–	–	2+
41	2+	2+	±	4+

1* *V. parahaemolyticus* F/674 maintained on artificial media
2* *V. parahaemolyticus*, Kanagawa positive, Thai source