INCIDENCE OF *VIBRIO PARAHAEMOLYTICUS* IN RELATION TO FEEDING HABIT OF FISHES

Vibrio parahaemolyticus, an enteropathogenic, facultative anaerobic halophile, has been implicated as the etiologic agent in a number of food poisoning outbreaks in many countries[1]. About 7 to 10% of cases admitted to the Infectious Diseases Hospital, Calcutta, are due to *V. parahaemolyticus*[2]. This marine bacterium is widely distributed in coastal waters, sediment, plankton and in a variety of organisms especially finfish and shell-fish inhabiting these environs[3]. The objective of the present study was to examine a variety of fishes from the aquatic environs of Porto Novo to investigate if feeding habit of fishes had any relation to the incidence of *V. parahaemolyticus*.

From November, 1977 to October, 1978, 1,047 fishes originating from brackish and neritic environs of Porto Novo, S. India (Lat. 11° 30′ N, Long. 79° 46′ E) were examined for the presence of *V. parahaemolyticus*. Sampling was performed at monthly intervals. Fish specimens caught in the field were immediately put into fresh polythene bags and transported to the laboratory in insulated containers (≤ 5° C). Generally, preference was given to average sized commercially important fishes with no visible signs of deterioration, injury or disease. Bacteriological examination was taken up within two hours after collection. At the laboratory, sand and detritus adhering to the fish were washed off with sterilised estuarine water. First swabs of the external surface (both flanks) were taken using sterile cotton tipped applicator sticks. Subsequently the entire gill surface was swabbed. An incision was then made in the vent region of the fish to expose the rectum and swabs of the faecal matter were taken. The swabs were inoculated in 10 ml glucose salt teepol broth and incubated for 6 hr at 37° C. A loopful of culture was then streaked on thiosulphate citrate bile salt sucrose agar and incubated for 18 hr at 37° C. Representative 'typical' green presumptive *V. parahaemolyticus* colonies[4] were picked up from positive plates and maintained on sea water yeast extract agar slopes. Identification was confirmed at the end of each month using the minimal diagnostic tests as advocated by Hugh and Sakazaki[5].

Based on their gut contents, fishes were broadly classified into four major feeding groups namely, carnivores, planktivores, omnivores and detritus feeders. Thirtyseven fishes, however, could not be categorised into any of these feeding types since their gut contents were completely digested. This group was designated as unidentified. Major groups of fishes examined among the carnivores were Silver bellies (Leiognathidae), Clupeoids (Chirocentridae, Clupeidae and Engraulidae), Catfishes (Tachysuridae), Ribbon fishes (Trichiuridae), Drummers and Croakers (Sciaenidae), Crescent perches (Theraponidae), Pomfrets (Stromateidae), Rabbit fishes (Siganidae), Lizard fishes (Synodidae) and Thread fins (Polynemidae). In the planktivores, the fishes sampled could be categorised under three groups, *viz.*, Indian Mackerel (Scombridae), Sardines (Clupeidae) and Milk fish (Chanidae) while in the omnivores the fishes examined comprised of *Oxyurichthys* sp. (Gobiidae), Tilapia and Pearl spots (Cichlidae). Detritus feeders were solely comprised of Mullets (Mugilidae).

Of the 1,045 fishes examined in the present study, 385 (36·8%) were positive for *V. parahaemolyticus*. Our results are in conformity with the findings of De *et al.*[2] which showed that 35·2% of sea water fishes in Calcutta environs harboured *V. parahaemolyticus*. A still higher isolation rate (47·3%) of *V. parahaemolyticus* in freshly caught fishes from lake Togo and adjoining lagoons (West Africa) has been reported[6]. However, investigations conducted in Canadian maritime regions[7] and in fish and shell-fish marketed in Netherlands[8], imply that *V. parahaemolyticus* was practically absent. These contrastive results from different geographical areas suggest a marked preponderance of *V. parahaemolyticus* in aquatic organisms, especially in finfish and shell-fish, inhabiting tropical systems.

Among the four feeding groups, *V. parahaemolyticus* was isolated from 56·3% of the detritus feeders while in carnivores, planktivores and omnivores, it was relatively less (Table I). Detritus feeders ingest bottom mud and sediment from which food items like benthic organisms and decaying flocculent deposits are separated by certain structural adaptations. Ecological studies in Chesapeake Bay[4] revealed the ubiquitous presence of *V. parahaemolyticus* in sediments even during winter months when *V. parahaemolyticus* counts in water and plankton were below detectable levels. Other investigators[9,10] also observed the pronounced predilection of *V. parahaemolyticus* for sediments and bottom mud. Studies on the seasonal distribution of *V. parahaemolyticus* at Porto Novo and the report of Manavalan *et al.*[11] have shown that sediments provide the most conducive ecological niche for this halophile in estuarine and mangrove environs of Porto Novo. In the present study, detritus feeders were solely composed of Mullets (Mugilidae). Mullets are known to feed on detritus, including mud and sand, and microalgae[12]. The same author has also suggested that much of the unrecognizable 'detritus' could be bacteria and protozoa. A recent study[13] on the gastro-intestinal microflora of *Liza dussumieri* (Mugilidae) has established the generic predominance of vibrios in the gut of this species. According to Moriarty[14], the Grey Mullet (*Mugil cephalus*) is capable of selecting small particles of sand rich in organic matter, especially algae and bacteria. All these findings clearly show a larger bacterial intake by detritus feeders than other feeding groups. It is therefore not surprising that *V. parahaemolyticus* could be isolated in greater frequency from detritus feeders. The results of this study indicating higher incidence of *V. parahaemolyticus* in detritus feeders may be attributed to their feeding habit.

The percentage incidence of *V. parahaemolyticus* in different feeding groups of fishes were tested employing the statistic 'd' of Bailey[15] and the results are

TABLE I

Incidence of V. parahaemolyticus *in different feeding groups of fishes and regions of isolation*

Feeding group	No. of samples examined	No. of samples positive (Percentage positive)	Regions of positive isolation: External surface	Gills	Faeces
Carnivores	690	252 (36·5)	83	55	143
Planktivores	156	49 (31·4)	11	41	42
Omnivores	45	12 (26·7)	6	3	8
Detritus feeders	119	67 (56·3)	17	46	64
Unidentified	37	5 (13·5)	3	1	2
Total	1047	385 (36·8)	120	146	259

TABLE II

Comparison of the incidence (percentage) of V. parahaemolyticus *in fishes with different feeding habits*

Sl. No.	Feeding habit	Feeding habit in comparison	Observed statistic '*d*' value	Significance P = 0·05
1.	Carnivores	Planktivores	1·2052	Not significant
		Omnivores	1·3365	Not significant
		Detritus feeders	4·0784	Significant
2.	Planktivores	Omnivores	0·6093	Not significant
		Detritus feeders	4·1414	Significant
3.	Omnivores	Detritus feeders	3·3902	Significant

presented in Table II. It is discernible that the incidence of *V. parahaemolyticus* was significantly higher ($P = 0 \cdot 05$) in detritus feeders than in other feeding groups.

In all four feeding groups, *V. parahaemolyticus* could be isolated more frequently from faecal samples of fishes than from the external surface or gills (Table I), suggesting that the gastro-intestinal tract of fishes may provide a suitable niche for their proliferation. The rich nutrient store available in the gut of fishes[16] may also aid the colonization of this pathogen especially during periods of unfavourable environmental conditions. Kaneko and Colwell[4] have postulated that *V. parahaemolyticus* can survive low temperatures of winter in sediments and in association with, or in, shell-fish and scavenger fish such as gobies. The results of the present study aver with the above observations, since it appears that the gut of fishes might serve as a 'provisional' environment to tide over unfavourable conditions like hypersalinity (tropical systems) and low temperatures (temperate systems). In planktivores, isolation of *V. parahaemolyticus* from the gills was quite high (Table I). The association of *V. parahaemolyticus* with zooplankton has been well documented[17]. The gills of planktivores are suitably developed to sieve plankton from water and such contact may result in *V. parahaemolyticus* adhering to the mucus of gill surface, resulting in the greater incidence of this pathogen in this region.

V. parahaemolyticus caused gastroenteritis, is less frequent in India compared to Japan since food items are well cooked and spiced here. However, chances of cross contamination *via* kitchen utensils or by handling may result in infection especially while handling mullets, the potential reservoirs of this pathogen. Degutting fishes immediately after the catch and washing them well would be advisable precaution to reduce the load of *V. parahaemolyticus* in such contaminated fishes.

We are extremely grateful to Dr. P. Sitarami Reddy for the help rendered in the identification of fishes and for statistical interpretation of the data. Financial assistance from ICAR and UGC are gratefully acknowledged.

Centre of Advanced Study in Marine Biology,
Porto Novo 608 502,
Tamil Nadu, *June* 11, 1979.

R. NATARAJAN.
G. BALAKRISH NAIR.
MARTIN ABRAHAM.

1. Miwatani, T. and Takeda, Y., *Vibrio parahaemolyticus, A Causative Bacterium of Food Poisoning*, Saikon Publishing Co., Ltd., Tokyo, 1976.
2. De, S. P., Banerjee, M., Deb, B. C., Sengupta, P. G., Sil, J., Sircar, B. K., Sen, D., Ghosh, A. and Pal, S. C., *Indian J. Med. Res.*, 1977, **65**, 21.
3. Natarajan, R., Martin Abraham, Ravi Gautam and Balakrish Nair, G., *Proceedings of the International Sym. on Environmental Agents and Their Biological Effects*, Int. S-2-59, 1978.
4. Kaneko, T. and Colwell, R. R., *J. Bacteriol.*, 1973, **113**, 24.
5. Hugh, R. and Sakazaki, R., *J. Conf. Public Health Lab. Directors*, 1972, **30**, 133.
6. Bockemuhl, J. and Triemer, A., *Bull. World Health Organ.*, 1974, **51**, 353.
7. Varga, S. and Hirtle, A., *J. Fish. Res. Board Can.*, 1975, **32**, 541.
8. Kampelmacher, E. H., Mossel, D. A. A., Van Noorle Jansen, L. M. and Vincentie, H., *J. Hyg.*, 1972, **68**, 189.
9. Chun, D., Chung, J., Seol. S. Y. and Tak, R., *Am. J. Trop. Med. Hyg.*, 1974, **23**, 1125.
10. Thompson, C. A., Vanderzant, C. and Ray, S. M., *J. Food Science*, 1976, **41**, 117.
11. Manavalan, S., Dhevendaran, K., Vanajakumar and Selvakumar, N., *Curr. Sci.*, 1977, **46**, 821.
12. Thomson, J. M., *Aust. J. mar. Freshwat. Res.*, 1954, **5**, 469.
13. Mary, P. P., *Ph.D. Thesis*, Annamalai University, India, 1977.
14. Moriarty, D. J. W., *J. exp. mar. Biol. Ecol.*, 1976, **22**, 131.
15. Bailey, N. T. J., *Statistical Methods in Biology*, The English Language Book Society, London, 1959.
16. Doudoroff, M., *J. Bacteriol.*, 1942, **44**, 461.
17. Kaneko, T. and Colwell, R. R., *Appl. Microbiol.*, 1975, **29**, 269.