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Monitoring of *Vibrio parahaemolyticus* in Aquaculture Ponds, Kerala, India

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Abstract: ***Qualitative and quantitative presence of the pathogenic bacterium V. parahaemolyticus in extensive and modified extensive Penaeus monodon culture systems on the southwest coast of Kerala, was monitored during three shrimp culture seasons. Annual mean V. parahaemolyticus in water constituted 6.7%. Percentage distribution of V. parahaemolyticus in sediment was the highest during premonsoon (90.6%) and the lowest during postmonsoon (89.4%), with monsoon registering 90.4%. Annual mean percentage in sediment was 90.1%. In shrimp, V. parahaemolyticus load during the three seasons were 3.8, 2.1 and 3.1%, with an annual mean of 3.1%. Percentage distribution of V. parahaemolyticus in water, in modified extensive system during the three seasons were 1.9, 5.4 and 13.5%, respectively. In the present study, both in extensive and modified extensive systems, V. parahaemolyticus registered the highest load in sediment throughout the three seasons. In both extensive and modified extensive systems V. parahaemolyticus showed an increasing trend up to the end of each culture season. In the present study, in both systems distribution of V. parahaemolyticus showed an increasing trend with increase in salinity. In extensive system, in shrimp and in modified extensive system, in water and shrimp, distribution of V. parahaemolticus was significantly negatively correlated with sand content (P < 0.05) and positively correlated with silt (P < 0.05).***

Keywords: *Vibrio parahaemolyticus*, extensive system, modified extensive system, *Penaeus monodon*, shrimp culture.

1. Introduction

Microbes are very important and have a critical role in aquaculture systems, at both hatchery and grow-out stages, because water quality and disease control are directly related and closely affected by microbial activity [1]. Outbreaks of virulent diseases in shrimp culture practices are attributable to a combination of factors involving infectious agents, environmental deterioration and stress due to intensification [2].

Microbial pollution of aquatic environment increases public health risk, especially when such contaminated water is used as a source of potable water and for fish and shellfish farming [3]. Microbial quality of shrimp culture systems, without any doubt, is a significant limiting factor in the success of shrimp aquaculture. Equally important is its bearing on public health.

Shrimp processors have been facing problem of rejection of their produce due to the presence of human pathogenic *Vibrio* spp. [4]. Contamination of hard skeleton of crustaceans and shells of bivalve molluscs with *Vibrio* is recognized as one of the cause of wound and blood infections following laceration of the skin sustained during handling of shellfish [5][6].

Vibrio parahaemolyticus is a gram negative, halophilic bacterium distributed in the temperate and tropical coastal waters and is a leading cause of food borne gastroenteritis [7]. In India, occurrence of *V. parahaemolyticus* in fish and aquatic environments has been reported by several workers [8] [9] [10]. *V. parahaemolyticus* count of 2.4 x10^4 MPN g^{-1} at the time of harvest was reported [11]. *V. parahaemolyticus* was isolated from the haemolymph of WSV infected shrimp collected from extensive, modified extensive and semi-intensive ponds in Visakhapatnam and East Godavari districts, Andhra Pradesh [12].

V. parahaemolyticus was detected in shrimp farms located at Tuticorin, Tamil Nadu [13]. The incidence of various human pathogenic bacteria in homemade shrimp feeds used in some farms in India was done [14]. The results showed that farm made feeds had high incidence of various human pathogens such as *V. parahaemolyticus and V. cholerae*.

The present study was undertaken to monitor the occurrence of *V. parahaemolyticus* of pond reared tiger prawn, *P. monodon*, pond water and sediment. In addition, the physicochemical characteristics of pond water and sediment were studied for checking whether there is any significant correlation between the changes in water/sediment quality and microbial load of shrimp and shrimp pond environment.

2. Materials and Methods

For present study six ponds growing *P. monodon* were selected from Kollam district, Kerala state (9.28'45º N and 76.28'0º E). In two ponds, (0.7 ha each), located at Munrothuruthu, fed with water from the Ashtamudi lake, extensive type of culture (stocking density = 5 m^{-2}) was practiced. In four ponds (0.4 to 0.6 ha each), two located at Mayyanadu, all fed with water from the Paravoor Kayal, modified extensive type of farming (stocking density =10 m^{-2}) was done. In these ponds pellet feed was given, thrice a day.

Shrimp specimens, water and sediment samples were collected aseptically from the six ponds for microbiological analyses. The samples were brought to the lab in ice baskets. The samples were properly prepared for further detailed studies, as soon as possible and always within three hours since collection. The samples were analysed for *Vibrio parahaemolyticus*. Suspected colonies were purified and confirmatory tests were done. For detection of *V. parahaemolyticus*, all media contained 3% NaCl. For enrichment APW with 3% NaCl was used. Broths were

incubated at 37°C for 16-18 h in a conical flask. After incubation, a loop full of culture was streaked on pre-set TCBS (contained 3% NaCl) agar plates and incubated at 36±1°C for 18-24 h. Typical *V. parahaemolyticus* colonies are round (3-5 mm), green or blue-green with green or blue centre.

Inoculated the suspected culture to TSI (Triple Sugar Iron) and KIA (Klingler Iron Agar) agar slants both containing 3% NaCl by stabbing the butt and streaking on slant. Incubated for 24 h [15]. In TSI, typical reaction of *V. parahaemolyticus* was indicated by alkaline slant (red) and acid butt (yellow). Further, there was no H_2S production. In KIA, *V. parahaemolyticus* gave alkaline slant (red) and acid butt (yellow) without H_2S production. If these tests were +ve, further confirmation was done by Gram staining (*V. parahaemolyticus* is Gram-ve, short or curved rods) and motility test (*V. parahaemolyticus* is motile), MRVP test (*V.parahaemolyticus* is VP–ve), Indole test (*V. parahaemolyticus* is indole +ve) and fermentation of sugars (*V. parahaemolyticus* is glucose, mannitol and arabinose +ve and sucrose, inositol -ve). Oxidase test (*V. parahaemolyticus* is oxidase+ve) and H & L glucose O/F test in which a yellow colour, which indicates the fermentation reaction and no gas. Further, salt tolerance test and catalase test was carried out. *V. parahaemolyticus* is catalase+ve. Standard methods were adopted for the analysis [16] [17] [18] [19] [15] and US FDA: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm, and Online version, Official Methods of Analysis, 18th ed., Revision 2, 2007http://www.eoma.aoac.org/.

Water temperature, pH and salinity (‰) were recorded at the site itself using a Celsius thermometer of $\pm$ 0.5°C accuracy, portable pH meter of $\pm$ 0.1 accuracy [Model No. ip (1-198107) RI, USA], and portable refractometer (Erma Inc., Tokyo), respectively. Dissolved oxygen content (DO, mg l^{-1}) [20], dissolved carbondioxide concentration (CO_2, mg l^{-1}) [21], hydrogen sulphide (H_2S, mg l^{-1}), total alkalinity (mg l^{-1}) , total solids (TS, mg l^{-1}), total dissolved solids (TDS, mg l^{-1}), total suspended solids (TSS, mg l^{-1}), calcium hardness (mg l^{-1}) [22], total hardness (mg l^{-1}) [23] and ammonia concentration (NH_3, mg l^{-1}) [24] were estimated employing standard methods. The difference between total and Ca-hardness was reckoned as Mg-hardness. For spectrophotometric assays, a dual beam spectrophotometer (Model UV2-100, UNICAM, UK) was used.

Sediment samples were collected using a PVC corer of length 25 cm and diameter 7 cm. The sediment samples were transferred into clean polythene bags and brought to the laboratory, pooled, air dried and sieved (sieve number-62 µm) before analysis. The temperature of the sediment was noted using a Celsius thermometer (calibrated before each collection) as soon as the corer was lifted out of water. Organic carbon content were expressed in percentage [25]. Soil texture was determined by the sieve and pipette method [26]. pH was measured [22] using a handheld pH meter (Model ip (1-198107) RI USA) calibrated before each set of measurement. Statistical significance of associations (dependence) of estimated microbial variables among themselves and between them and hydrobiological variables were tested using correlation analysis. Relevant theoretical inputs for statistical analyses were adopted [27] and analyses were done using "statistiXL 1.8" package.

3. Results and Discussion

Distribution of *V. parahaemolyticus* in extensive and modified extensive shrimp culture system are presented in Table 1; Fig. 1. The results of statistical analyses are included in Tables 2 and 3.

In extensive system, the highest percentage of *V. parahaemolyticus* in water was noted during monsoon (7.6%) and the lowest during premonsoon (5.5%), with postmonsoon registering 7.5%. Annual mean *V. parahaemolyticus* in water constituted 6.7%. Percentage distribution of *V. parahaemolyticus* in sediment was the highest during premonsoon (90.6%) and the lowest during postmonsoon (89.4%), with monsoon registering 90.4%. Annual mean percentage in sediment was 90.1%. In shrimp, *V. parahaemolyticus* load during the three seasons were 3.8, 2.1 and 3.1%, with an annual mean of 3.1%.

Percentage distribution of *V. parahaemolyticus* in water, in modified extensive system during the three seasons were 1.9, 5.4 and 13.5%, respectively, for premonsoon, monsoon and postmonsoon seasons with an annual mean of 3.5%. In sediment, it was 97.8, 92.1 and 84.8%, respectively, with an annual mean of 95.9 %. In shrimp, percentage distribution of *V. parahaemolyticus* in modified extensive system during the three seasons were 0.3, 2.5 and 1.7%, with an annual mean of 0.6%.

One noteworthy feature was that *V. parahaemolyticus* in water ($P < 0.01$), sediment ($P < 0.05$) and shrimp ($P < 0.01$) was significantly positively correlated with salinity in extensive system (Table 2). In extensive system, *V. parahaemolyticus* in water ($P < 0.05$) and shrimp ($P < 0.01$) showed significant positive correlation with clay content. In extensive system, in shrimp and in modified extensive system, in water and shrimp distribution of *V. parahaemolyticus* was significantly negatively correlated with sand content ($P < 0.05$) and positively correlated with silt ($P < 0.05$) (Table 3).

In the present study, both in extensive and modified extensive systems, *V. parahaemolyticus* registered the highest load in sediment throughout the three seasons. This might be because of the prolonged survival of bacteria in sediment, which offers more favourable chemical and biological environments [28] [29][30]. In Australian shrimp farms, the load of vibrios was 50 to 100 times higher in pond sediment than in pond water [31]. In environmentally friendly ponds growing *P. monodon*, reported higher *Vibrio* count in sediment than in water and shrimp [32]. In conformity with this, higher load of bacteria in bottom soil than in water was reported from shrimp ponds [32]. In El-Qanater fish farm, noted higher bacterial counts in sediment than in water [33].

In both extensive and modified extensive systems *V. parahaemolyticus* showed an increasing trend up to the end of each culture season. Smith [34] noted that levels of

vibrios in pond sediment increased during grow-out as temperature and input of feed increased. A maximum *V. parahaemolyticus* count of 3.7 $x10^3$ cfu ml^{-1} in water, 5.5 $x10^3$ cfu g^{-1} in sediment and 1.9 $x10^2$ cfu g^{-1} in shrimp was noted on the 150^{th} day of culture from shrimp culture environments by Dalmin et al. [35]. A maximum *V. parahaemolyticus* count in sediment at 150^{th} day of culture ($5.5x10^7$ cfu g^{-1}) and the minimum at 25^{th} day of culture ($1.3 x10^7$ cfu g^{-1}) was noted in an aquaculture pond by Anand Ganesh et al. [36]. These are in conformity with the present observations.Decomposition of excess feed and algal die-off result in proliferation of vibrios [37]. High organic load in modified extensive culture system is also known to increase *Vibrio* count [38]. In the present study, in both systems distribution of *V. parahaemolyticus* showed an increasing trend with increase in salinity. Influence of salinity on the survival of *V. parahaemolyticus* is well documented [39] [40]. *V. parahaemolyticus* from Tuticorin coastal environments, was isolated more frequently during summer; its density declined during monsoon season [41]. While studying the microbial quality and physicochemical parameters of a brackishwater shrimp culture pond, Sanjeev [11] observed that salinity varied from 1.83 to 24.58‰ and that *V. parahaemolyticus* count of water was the maximum during February to April when salinity was high and it was absent from July to November, when the salinity of water was minimum. Vibrios are autochthonous to saline water and hence the recovery of *Vibrio* spp. in both water and sediment from brackishwater ponds is quite natural [42]. In Cochin backwaters [43] and in Abu Dhabi coastal waters [44], the maximum bacterial count was noted during warmer months. So also did in extensive aquaculture ponds adjacent to Cochin backwaters [42], in Cochin backwaters [45], in old port, Bhavnagar coast [46] and in El-Qanatar fish farm, Cairo [33]. It is noteworthy here that, living conditions remaining favourable, microorganisms will quickly multiply during summer. The present results are in agreement with the foregoing.

4. Conclusion

V. parahaemolyticus is a human pathogen of marine origin and can be found in water, sediments, plankton, finfish and shellfish of coastal and estuarine environments and it is known to cause diarrhoea, gastroenteritis, wound infection, ear infection and secondary septicaemia in humans. Presence of this pathogen in substantial quantities in all three compartments (water, sediment and shrimp) of both extensive and modified extensive shrimp culture ponds, must be reckoned as a warning signal on the environmental deterioration of such ponds and on the high likelihood of precipitation of shrimp and human health hazards. Measures should be taken by the aquaculturists to maintain good microbial quality of ponds as their product being handled and consumed by mankind.

Table 1: Mean microbial load (given as bacterial count) in the three compartments, water (cfu ml-1), sediment (cfu g-1) and shrimp (cfu g-1) and shrimp (cfu g-1) and percentage distribution of various microbial loads in three compartments of extensive and modified extensive shrimp culture systems during three seasons of shrimp culture operation.

Bacterial Counts	Compartments	Extensive System				Modified Extensive System			
		Pre-monsoon	Monsoon	Post- monsoon	Mean	Pre- monsoon	Monsoon	Post- monsoon	Mean
V. parahaemolyticus	Water (cfu ml^{-1})	197	171	228	196	896	181	922	618
	Sediment (cfu g^{-1})	3217	2038	2725	2598	46463	3103	5788	16916
	Shrimp (cfu g^{-1})	136	46	95	88	163	85	117	118
		Extensive System				Modified Extensive System			
V. parahaemolyticus (%)	Water	5.5	7.6	7.5	6.7	1.9	5.4	13.5	3.5
	Sediment	90.6	90.4	89.4	90.1	97.8	92.1	84.8	95.9
	Shrimp	3.8	2.1	3.1	3.1	0.3	2.5	1.7	0.6

Table 2: Results of correlation analysis showing *r* values comparing various *V. parahaemolyticus* loads in water with hydrographical parameters of extensive (N = 20) and modified extensive (N = 40) shrimp culture system.

Exensive culture system			Modified extensive system		
Water	Sediment	Shrimp	Water	Sediment	Shrimp
0.107	0.071	0.08	-0.163	-0.057	-0.024
-0.138	-0.277	-0.348	-0.133	-0.071	-0.131
0.591#	0.540*	0.653#	0.246	0.019	0.144
-0.323	-0.382	-0.361	-0.159	-0.255	0.038
0.328	0.369	0.529*	0.189	0.341*	0.062
0.325	0.3	0.477*	0.223	0.139	0.159
0.355	0.362	0.296	0.448#	0.255	0.323*
0.151	0.104	0.218	-0.087	-0.072	0.024
0.395	0.428	0.634#	0.362*	0.11	0.216
0.426	0.479*	0.719#	0.375*	0.15	0.254
0.466*	0.395	0.559*	0.232	-0.055	0.065
0.127	0.276	0.472*	0.196	0.141	0.285
-0.137	0.239	0.193	0.034	0.081	0.148
0.18	0.225	0.454*	0.237	0.146	0.3
0.583#	0.557*	0.41	0.061	0.084	-0.173
-0.249	-0.212	-0.197	0.306	0.066	0.266
-0.312	-0.429	-0.342	-0.118	-0.202	-0.162
-0.04	-0.064	0.126	0.176	-0.009	0.082
0.467	0.41	0.605#	0.031	-0.076	0.17
***P* < 0.05; #*P* < 0.01**					

Table 3: Results of correlation analysis showing r values comparing V. parahaemolyticus loads with sedimentological parameters of extensive (N = 20) and modified extensive (N=40) shrimp culture system

	Extensive System			Modified Extensive System		
Compartments	Water	Sediment	Shrimp	Water	Sediment	Shrimp
Temp.	0.227	0.272	0.336	-0.027	0.029	0.16
pH	-0.393	-0.322	-0.246	-0.134	-0.025	-0.404*
OC	-0.129	0.152	0.091	-0.148	-0.055	-0.013
Sand	-0.302	-0.263	-0.525*	-0.394*	-0.267	-0.351*
Clay	0.537*	0.351	0.590#	0.136	-0.062	0.061
Silt	0.244	0.232	0.481*	0.397*	0.297	0.364*

* *P* < 0.05; # *P* < 0.01

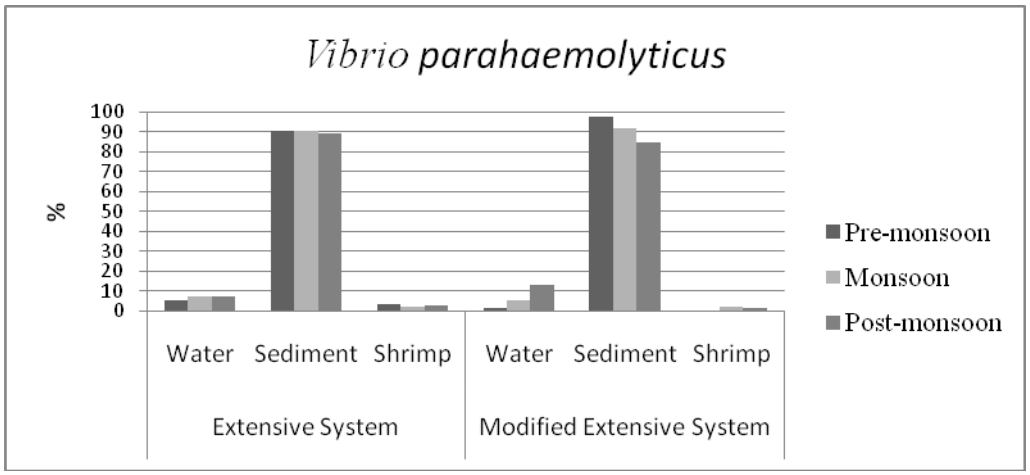

Figure 1: Seasonal variation in *V. parahaemolyticus* distribution in extensive and modified extensive *Penaeus monodon* culture systems

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