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Parasite Induced Vibriosis in *Stolephorus commersonii*

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Abstract: The attempt of the present study is to achieve an approach of the magnitude of parasite induced secondary infection estimation of *Vibrio*, *Salmonella* and *Pseudomonas* loads as well as the total heterotrophic bacteria using a battery of biochemical tests. The parasitic isopod *Nerocila phaiopleura* was collected from commonly available fish *Stolephorus commersonii* at Parangipettai, India. Parasites settled on the branchial and external body surface of the fish causes red coloured skin lesions, where secondary bacterial infections were detected. THB and *Vibrio* counts were higher in the infected hosts than in the healthy fish and it was found to 7.2×10^5 and 3.5×10^3 cfu g^{-1} in branchial regions and 5.4×10^5 and 2.3×10^3 cfu g^{-1} in the body surfaces, respectively. *Salmonella* counts were found as 6.0×10^3 and 3.6×10^1 cfu g^{-1} in branchial regions of the infected and uninfected fish and in body regions, 3.9×10^3 and 1.03×10^2 cfu g^{-1}, respectively. *Pseudomonas* counts were 7.0×10^2, 4.9×10^1 cfu g^{-1} in branchial regions and 6.2×10^2, 5.8×10^1 cfu g^{-1} in the body surfaces of the infected and uninfected fish. Statistical analysis of the results (t-test) showed high significant value ($p<0.05$).

Key words: Parasitic isopod, *Stolephorus commersonii*, bacterial infections, vibriosis

INTRODUCTION

Aquatic animals are continuously bathed in an aqueous suspension of microorganisms and their external surfaces are always in contact with them. Existence of pathogenic microbes in an aquatic environment is inevitable. Infections generally occur when the animal is immunologically incompetent either due to physical or biological stress such as parasitic attack. Thus, secondary infections are induced by opportunistic bacteria, which invade in to the body of parasitized organisms. Access and settlement of pathogenic microbes are facilitated through the injuries caused by parasites. In general, there are two types of secondary infections; an attack by the harmful organism is sometimes possible given the general debility due to the primary infection; the other is an active transfer of the pathogen by the parasite (Kabata, 1970).

The family Vibrionaceae is an indigenous group of bacteria in the biota of marine and estuarine environments, constituting 0.1-60% of the total heterotrophic bacteria (Simidu and Tsukamoto, 1985). *Vibrio* species are implicated as primary as well as secondary pathogens in fin and shellfish.

Freshly caught marine fish harbors a large number of bacteria on their skin, gill surface and intestine. Most of them are normally saprophytic but a few become pathogenic either after (parasitic injury) to the animal or when adverse environmental or physiological conditions prevail

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(Bisset, 1946; Schaperclaus, 1989). Fish are subjected to various diseases, some with infectious aetiology. More than 50 bacterial species have been reported to associate with diseases in freshwater and marine fishes. The greatest economic losses were induced by *Aeromonas hydrophila*, *Renibacterium salmonarum*, *Streptococcus* sp. (Austin and Austin, 1987; Rohovec, 1991). The most severe bacterial diseases encountered in Norwegian aquaculture were vibriosis mainly due to *V. salmonicida* (Holm and Jorgensen, 1987) and a furunculosis induced by *A. salmonicida* (Lunder and Hastein, 1990).

Incidence of infecting parasites like isopods may severe enough to produce open lesions on the fish, irritant to the epithelial layers. So, access of the smaller unicellular which can be quite destructive. Some studies suggested that fish parasites might be vectors for viral, bacterial and fungal pathogens. Nigrelli (1950) reported that the ectoparasite copepod *Ergasilus* sp. is the etiological agent for bacterial diseases in fish. Casack and Cone (1985) observed that the *Gyrodactylus avalonia*, carry pathogenic bacteria into fish and *A. punctata*, infect the fish secondarily at the site where the parasite *Argulus* sp. settle. Parasitic isopods and the copepod *Lernaea* sp. were reported transferring viruses causing dermal tumors in fish (Simidu and Tsukamoto, 1985). Overstreet and Howse (1977) reported that the haemorrhagic lesions produced by parasitic isopods are possible sites for secondary infections by microorganisms. Bullock (1971 and 1981) and Snieszko (1983) stated that infection might be communicable as long as infected fish are present in the environment with causative agent. A disease outbreak may occur at any time particularly when the fish is under stress.

Outbreak of bacterial diseases in fish subsequent to parasitic isopods infection is scarce with few studies carried out in India. As a result, we are interested to report the incidence of such a secondary bacterial infection in the fish *Stolephorus commersonii* Lacépède, 1803 (Actinopterygii; Clupeiformes; Engraulidae).

MATERIALS AND METHODS

Identification of Parasite

During the routine observation of *S. commersonii* in fish landings at Parangipettai (Lat. 11° 29'N; Long. 79° 46'E, India) an isopod parasite was observed on fish with an average length and weight of 11.6±0.21 cm and 9.68±0.14 g, respectively (Fig. 1). The parasite was identified as *Nerocila phaiopleura* Bleeker, 1857 (Crustacea; Isopoda; Cymothoidae). Incidence of isopods was maximal on the branchial and the body surface of the fish. At the site of parasitic settlement, skin

Fig. 1: *Nerocila phaiopleura* on *Stolephorus commersonii*

lesions were observed in the infected fish. The damaged area was red coloured and devoid of scales. The work was carried out at CAS in marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India in March, 2007.

Microbiological Analysis

Microbiological study was performed after removing 1 g of the injured skin from parasite infected and uninfected fishes. Tissues excised were separately homogenized with sterilized seawater and subjected for further microbiological investigations.

One milliliter of the serially diluted samples were used for the estimation of Total Heterotrophic Bacteria (THB) and *Vibrio* count (VC). *Salmonella* and *Pseudomonas* were also counted. THB was evaluated using Zobell marine agar, VC using thiosulphate citrate bile salts sucrose agar (TCBS), *Salmonella* in SS agar and *Pseudomonas* in centrimade agar. All mean counts were expressed as colony forming units g^{-1} (cfu g^{-1}). Isolates were purified and stored in nutrient agar slants fewer than 4°C. Bacterial strains isolated from the aforementioned media were identified according to Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974) based on their biochemical and physiological characteristics.

RESULTS AND DISCUSSION

In the parasitized fishes, THB, *Vibrio*, *Salmonella* and *Pseudomonas* loads were higher than the uninfected fishes (Table 1).

In the branchial region of the parasitized fish, THB was found to be 7.2×10^5 cfu g^{-1} whereas in healthy fish it was 1.2×10^3 cfu g^{-1} and the *Vibrio* loads were 3.5×10^3 and 1.2×10^1 cfu g^{-1}, respectively. *Salmonella* and *Pseudomonas* counts rose to 6.0×10^3 and 7.0×10^2 cfu g^{-1} in parasitized fish whereas they were 3.6×10^1 and 4.9×10^1 cfu g^{-1}, respectively in uninfected fishes.

The parasitized areas of the body surface of fish showed a THB value of 5.4×10^5 cfu g^{-1} whereas in uninfected specimens it was only $1.5\text{x}10^3$ cfu g^{-1}. The VC from the body surface was 2.3×10^3 and 2.1×10^2 cfu g^{-1} in infected and uninfected fish. The *Salmonella* counts were of 3.9×10^3 and 1.03×10^2 cfu g^{-1}, for *Pseudomonas* 6.2×10^2 and 5.8×10^1 cfu g^{-1}, in parasite infected and uninfected fish, respectively.

The *Vibrio* isolates were comprised of *V. angullarium*, *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*. The two dominant species were *V. angullarium* and *V. parahaemolyticus*. *Salmonella* and *Pseudomonas* isolates showed only *S. typhi* and *P. aeruginosa*.

A number of contaminations were reported resulting from eating *Vibrio* contaminated raw seafoods (Sanjeev and Stephen, 1994). The major contaminants are *V. parahaemolyticus*, *V. cholerae*, *V. vulnificus*, *V. fluvialis* and *V. alginolyticus*. The presence of human enteric organisms on marine food products clearly indicates the contamination of terrigenous origin.

Table 1: Total heterotrophic bacteria and *Vibrio* counts on infected and uninfected *Stolephorus commersonii*

Source	Region	Infected fishes (cfu g^{-1})	Uninfected fishes (cfu g^{-1})
THB	Branchial region	7.2×10^5	1.2×10^3
	Body surface	5.4×10^5	1.5×10^3
Vibrio	Branchial region	3.5×10^3	1.2×10^1
	Body surface	2.3×10^3	2.1×10^2
Salmonella	Branchial region	6.0×10^3	3.6×10^1
	Body surface	3.9×10^3	1.03×10^2
Pseudomonas	Branchial region	7.0×10^2	4.9×10^1
	Body surface	6.2×10^2	5.8×10^1

cfu g^{-1}: Colony forming units/gram

Ulcerations or external lesions in fish might have been caused by a number of factors other than bacterial infection induced by parasitic attack (Sindermann, 1979), such as polluted environment and other adverse influences. However, parasites also contribute significantly to the spread of microbial diseases. Parasitic crustaceans are the largest fish parasites, which cause considerable damages to their hosts. They were involved in the spread of *Lymphocystis* disease (Nigrelli, 1950), *Icthyophorrus* contamination (Mann, 1970) and other secondary microbial infections. Overstreet and Howse (1977) proposed that haemorrhagic lesions in the spotted gore parasitized by the cymothoid *Anilocra acuta*, were subjected to secondary infection. In the present study, cymothoid *N. phaiopleura* parasitizing *S. commersonii* paved the way for pathogenic germs.

The bacterial load involved in the microbial infection depends on the site infected. In the present study, a regional difference for bacterial proliferation was observed: THB, VC, *Salmonella* and *Pseudomonas* counts were higher in the host's branchial region than in the body surface in both infected and uninfected fish. This may be due to the direct exposure of gills to the outer environment. The higher THB, VC, *Salmonella* and *Pseudomonas* counts in the branchial region could be attributable to the severity of the lesions at that level, as reported in the Creole fish parasitized by *N. acuminata* (Rand, 1986). Also, the recorded frequent contamination of this area may be due to the respiratory water current that carries bacteria along with food materials and could facilitate bacterial invasion. Besides, the increased level of bacterial population may also lead to further infections.

Pathogenic microbes and parasites could damage the physiological and reproductive activities of the host fish (Ranjit Singh and Padmalatha, 1997; Vismanis and Kondratovics, 1997). The bacterial invasion in the branchial region reduces the respiratory area injuring the gill lamellae and affects respiration as well as excretion of nitrogenous materials and disturbs the osmotic balance (Rand, 1986; Meenashi, 1997; Ravichandran *et al*., 2001). Lesions induced by parasitic attack become the sites for secondary infections by opportunistic bacterial and fungal pathogens that combined with the parasitosis impacts, which cause deleterious effects on the physiology of the animal and leads to death.

The attempt of the present study is to achieve an approach of the magnitude of such a secondary infection counting the *Vibrio*, *Salmonella* and *Pseudomonas* loads as well as the total heterotrophic bacteria using a battery of biochemical tests. THB, VC, *Salmonella* and *Pseudomonas* counts were higher in the infected area of fish, vulnerable to attack by the microorganisms. The two dominant isolated *Vibrio* species are *V. angullarium* and *V. parahaemolyticus*. *V. angullarium* has been earlier reported as a potential fish pathogen by different authors (Kusuda, 1966; Hacking and Budd, 1971; Parker and Smith, 1984; Singh *et al*., 1996). Vibriosis in fish caused by *V. angullarium* is known to affect a wide variety of fish species from brackish water to seawater (Loganathan, 1985). Lightner (1977) reported that *V. parahaemolyticus* is among the causative organisms of *Vibriosis* in prawn and also associated with outer secondary infections. This species was also reported from cultured marine fishes (Sindermann, 1970; Raghukumar, 1971; Ravichandran *et al*., 2001). *V. parahaemolyticus* has not been specifically identified as a pathogen of marine fish although Kusuda (1966) has reported *Vibrios* causing ulcerous diseases in cultured marine fish with close similarity to intestinal inflammation in man caused by *V. parahaemolyticus*. According to the above results, *V. parahaemolyticus* may be considered as a significant pathogen involved in secondary infection. Harrell *et al*. (1976) demonstrated that isolates of *V. anguillarium* act as the most important aetiological agent of vibriosis. In the case of uninfected fish, THB and *Vibrio* counts were very low. This may be due to the healthiness of the fish, which will interfere with the contact between the microbe and fish, as a result of continuous secretion of mucus from the skin of the fish.

Salmonella has been reported in the gut of *Tilapia* and carps (Iyer and Shrivastava, 1989; Ogbondeminu, 1993) and as a fish pathogen in salmon (Austin *et al*., 1982) as reposted in the present study. Occurrence of *Pseudomonas* in the infected fish was witnessed by Eddy and Jones (2002), Simidu and Tsukamoto (1985) and *Salmonella* by Karunasagar *et al*. (2004). *Pseudomonas* exists

throughout the aquatic environment associated with both healthy (Evelyn and McDermott, 1961; Bullock and Sniesko, 1969) and unhealthy fish. Presence of *Klebsiella pneumoniae* in a badly damaged fin and tail has been recorded by Austin and Austin (1999). *P. anguilliseptica* and *P. fluorescens* were reported as a causative agent of red spot and generalized septicaemia in rainbow trout and most of the marine species (Austin and Austin, 1999). Statistical analysis of the results obtained from the present study showed a high significance of $p<0.05$.

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