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Swollen hindgut syndrome (SHG) of tiger shrimp *Penaeus monodon* (Crustacea, Malacostraca, Penaeidae) post larvae: Identification of causing pathogenic bacteria and their sensitivity to some antibiotics

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Abstract. Swollen Hindgut syndrome (SHG) of black tiger shrimp *Penaeus monodon* Fabricius, 1798 post larvae has been reported from Bangladesh shrimp hatcheries in recent years. At present SHG is a budding problem causing significant economic loss to the shrimp hatcheries in Bangladesh. Unlike the rapid mortalities associated with viral disease such as white spot syndrome and yellow head virus, progression of SHG is gradual leading to low level mortalities without affecting swimming activity. The sign of SHG are a bloated or swollen in hind gut area, with the posterior digestive tract convoluting through the last abdominal segment. This syndrome tends to occur at later PL stages, typically after PL10. Two types of *Vibrio* spp. were isolated from the swollen hindgut syndrome post larvae, identified as *Vibrio harveyi* (Johnson and Shunk 1936)and *Vibrio alginolyticus* (Miyamoto, Nakamura & Takizawa 1961). Among these *V. alginolyticus* was dominant to *V. harveyi*. The bacterial isolates showed sensitive to oxytetracycline (OTC), norfloxacin and ciprofloxacin and resistant to penicillin, ampicillin and amoxycillin. The luminous *V. harveyi* showed resistant to many antibiotics and susceptibility to only two drugs. The cause of swollen hindgut syndrome (SHG) was probably bacterial infections and poor water quality, possibly heavy metal i.e. iron, the presence of toxic substances from chemical prophylactics and low quality or diseased nauplii.
Key Words: Swollen hindgut syndrome, *Vibrio* spp. antibiotic, *Penaeus monodon*.

সারাংশঃ সাম্প্রতিক বছরগুলোতে বাংলাদেশের বাগদা চিংড়ি হ্যাচারীতে সোলেন হিন্ডগার্ট রোগের প্রকোপ দেখা যাচ্ছে। বর্তমানে সোলেন্ড হিন্ডগার্ট রোগটি উদিয়মান সমস্যা হিসাবে দেখা দিচ্ছে যা চিংড়ি হ্যাচারীগুলোকে অর্থনৈতিকভাবে ক্ষতিগ্রস্থ করছে। চিংড়ির ভাইরাস ডিজিস যেমনঃ হোয়াইট স্পট ও ইয়োলো হেড এর মতো সোলেন হিন্ডগার্ট রোগে চিংড়ির মড়ক লাগেনা কিন্তু চিংড়ির উৎপাদন রেট কমে যায়। সোলেন হিন্ডগার্ট-এর সিন্ড্রোম হচ্ছে সোলেন হিন্ডগার্ট রিজিয়নের লাষ্ট সেগম্যান্ট ফুলে যাওয়া। এই সিন্ড্রোমটা প্রধানত পি.এল. ১০ এর পরে দেখা যায়। দুই ধরনের ব্যাক্টেরিয়া যথাঃ ভিবরিও এলজিনোলাইটিকাস এবং ভিবরিও হারভেই সোলেন হিন্ডগার্ট আক্রান্ত পি এল এ পাওয়া গেছে। এর মধ্যে ভিবরিও এলজিনোলাইটিকাসটি ভিবরিও হারভেই এর চেয়ে বেশী পাওয়া গেছে। এন্ট্রিবায়োটিক সেনসিটিভিটি টেস্টে দেখে গেছে যে, ব্যাক্টেরিয়াগুলো অক্সিটেট্রাসাইক্লিন, নরফ্লোক্সাসিন এবং সিপ্রোফ্লোক্সাসিন সেনসেটিভ এবং এমপিসিলিন ও এমোক্সিসিলিন রেজিষ্টেন্ট। সোলেন হিন্ডগার্ট রোগটি সম্ভবত ব্যাক্টেরিয়া জনিত রোগ এবং খারাপ হ্যাচারী ব্যবস্থাপনা বিশেষতঃ হেভিমেটাল এবং টক্সিক সাবস্টেন্স-এর জন্য হতে পারে।
মলশব্দ: সোলেন হিন্ডগার্ট, ভিবরিও, বাগদা চিংড়ি, এন্টিবায়োটিক।

Introduction. Culture of tiger shrimp *Penaeus monodon* Fabricius, 1798 is one of the most profitable business in aquacultures sector in Bangladesh. Since the 2000, shrimp farming has played a key role in national economy. Bangladesh stood seventeenth by volume (23000 MT) and eighth by value (US$ 246.6 million) of shrimp (wild and cultured) exports in the year 2002 (WB, NACA, WWF and FAO 2002). Commercial culture of shrimp involves stocking of shrimp seeds (post larvae) in the ponds rearing them to a marketable size and harvesting. Shrimp farming is practiced in Bangladesh as monostock of the black tiger shrimp. The entire culture is almost dependent on the hatchery reared post larvae. With the rapid expansion of shrimp grow out ponds, the hatchery industry has progressed rapidly during the last few years. At present, there are 56 shrimp

hatcheries in Bangladesh, among them 45 are running (Uddin & Kader 2006). However, failures in shrimp production occurred due to different diseases and caused by bacteria, viruses and fungi. Bacteria are the most common biological agents in the aquaculture and it is also known that marine crustaceans can be infected by one or more type of bacteria. In many shrimp producing countries, *Vibrio* and *Aeromonas* are considered as the most common and significant infectious pathogens (Lightner 1996, Moriarty 1997, Vaseeharan et al 2005).

At present swollen hindgut syndrome (SHG) is a budding problem in the shrimp hatcheries in Bangladesh. Shrimp farmers avoid stocking seeds showing SHG which results in severe losses to shrimp hatchery owners. However SHG does not show the mass mortality of *P. monodon* larvae like WSSV disease. Shrimp seeds with normal hind gut have a swollen hindgut with or without melanisation. This condition is also referred to as hindgut expansion. In the affected seeds, the rhythmic movements in the rectal region are affected resulting in difficulty in expelling the faecal pellets (Uma et al 2008). The main objective of this study is to isolate and identify the bacterial pathogen from post larvae with SHG and their sensitivity to some antibiotics.

Material and Method. Post larvae with SHG were collected from two commercial shrimp hatcheries located at the Kolatoly hatchery zone, Cox's Bazar (Figure 1). The samples were transported in live condition in aerated polythene bags and brought to shrimp disease diagnosis laboratory, Institute of Marine Sciences & Fisheries, University of Chittagong. The samples were observed microscopically to confirm the presence of SHG syndrome. Post larvae showing typical changes specific to SHG were selected and subjected to bacteriological examination. The samples were surface sterilized with 25 ppm of sodium hypochlorite for 2 minutes before rinsed in filtered seawater and their guts were separated. 1 gram sample of post larvae from each hatchery were taken separately and placed in individual sterile test tube and homogenized in 1mL filtered seawater using a sterile glass rod. The homogenate was then serially diluted up to 10^{-3} in filtered seawater. 1 mL from each dilution was plated by pipette onto thiosulphate citrate bile salts sucrose (TCBS) agar media. After solidifying of the media the plates were inverted and placed in incubator to incubate at 35ºC for 48 hours. For isolation, the suspected colonies were repeatedly streaked on TSA medium (Oxoid, UK) and then the pure cultures were stored in nutrient agar slants in a refrigerator at 4^0C. The bacteria were examined to standard morphological, physiological and biochemical tube and plate tests according to the procedures of Colwell et al (1984) and Holt et al (1994). The sensitivity of the isolates to various antibiotics were tested following the method of Vaseeharan et al (2005).

Figure 1. Map showing sampling area of Kolatoly hatchery zone, Cox's Bazar.

Results and Discussion. Swollen hindgut syndrome was noticed in many shrimp hatcheries situated in Cox's bazaar, Bangladesh. The disease was observed in later PL stages of shrimp and was more predominant in PL_{15}-PL_{25}. The clinical signs of the infected animals were bloated or swollen in hind gut area, with the posterior digestive tract convoluting through the last abdominal segment (Figure 2 and 3).

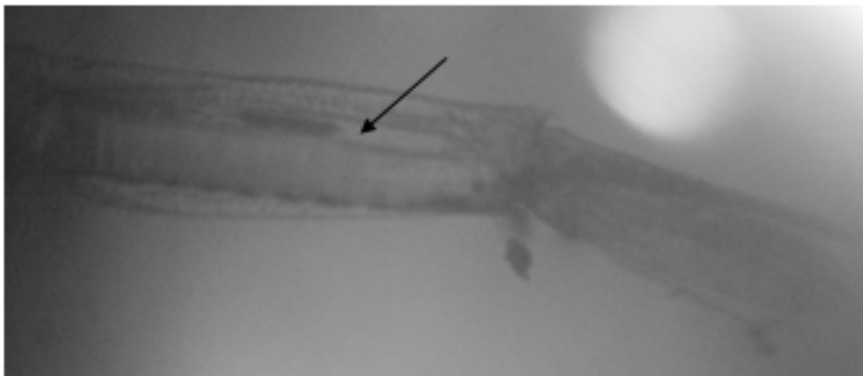

Figure 2. Normal hindgut of a healthy post larva of *P. monodon* (Magnification 10X).

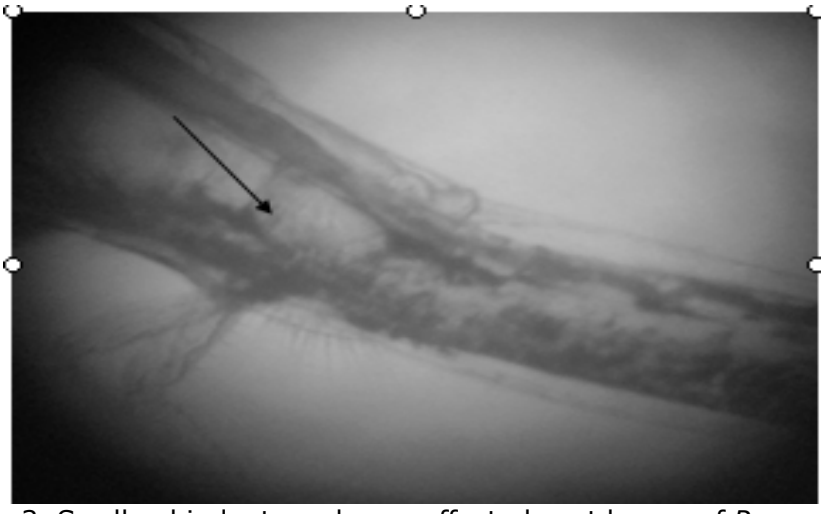

Figure 3. Swollen hindgut syndrome affected post larvae of *P. monodon* (Magnification 10X)**.**

However, this problem does not show the mass mortality of *P. monodon* larvae like WSSV disease. It has negative effects on digestion and excretion and significantly reduced the production of PL. The causes of this syndrome are not known, but the present study found two gramnegative bacteria was responsible for this syndrome. Two species of *Vibrio* were isolated from the affected PL, identified as *Vibrio alginolyticus* and *Vibrio harveyi* (Table 1). Among these *V. alginolyticus* was dominant to *V. harveyi*. Lightner (1996) reported that *V. alginolyticus* is very common in the marine environment. Uma et al (2008) found *V. harveyi*, *V. alginolyticus* and *Vibrio campbellii* (Baumann, Baumann and Mandel 1971) in swollen hindgut affected post larvae in India. Karunasagar (1994) reported that mass mortalities of *P. monodon* associated with *Vibrio* spp. have been observed in shrimp hatcheries in India. Lavilla-Pitogo et al (1990) reported that larval prawns are particularly susceptible to *V. harveyi*. Nash et al (1992) reported that pathogenic strains of *V. harveyi, Vibrio vulnificus* (Reichelt, Baumann and Baumann 1979)*, Vibrio parahaemolyticus* (Fujino, Okuno, Nakada, Aoyama, Fukai, Mukai and Ucho 1951) have caused massive epidemics in Thailand. Baumann & Baumann (1981) reported that *V. alginolyticus* is very common in coastal waters of temperate and tropical regions. Nash et al (1992) reported that *V. alginolyticus* is to be pathogen for *P. monodon* larvae.

Drug sensitivity studies revealed that all the bacterial isolates were sensitive to Oxytetracycline (OTC), Norfloxacin and Ciprofloxacin and resistant to Penicillin, Ampicillin and Amoxycillin (Table 2). Hence, we can say the causes of swollen hindgut syndrome may be bacterial and also poor water quality i.e. iron toxicity. The presence of toxic substances from chemical prophylactics, and low quality/diseased nauplii are also another possible factor for this disease.

Table 1

Morphological and biochemical characteristics of *Vibrio* spp. isolated from swollen hindgut syndrome of *Penaeus monodon* post larvae

Tests	*V. alginolyticus*	*V. harveyi*
Gram stain	-	-
Growth on TCBS	Y	Y
Motility	+	+
Oxidase reduction	+	+
Catalase reduction	+	+
O/F test	F	F
Arginine dihydrolase	-	-
Lysine decarboxylase	+	+
Ornithine decarboxylase	+	+
Methyl red test	+	+
Voges proskauer	+	-
Indole production	+	+
Nitrate reduction	+	+
Citrate utilization	+	+
Gelatinase	+	+
Esculin hydrolysis	+	-
Sensitivity to O/129 (10 µg)	+	+
O/129 (150 µg)	+	+
Growth at 4^0C	-	-
Growth at 42^0C	-	-
Growth NaCl (%)		
0	-	-
0.5	+	+
1	+	+
3	+	+
6	+	+
8	+	+
10	-	-
Acid production from		
Arabinose	-	-
Cellobiose	-	+
Galactose	+	+
Glucose	+	+
Inositol	-	-
Lactose	-	-
Mannitol	+	+
Sorbitol	+	+
Sucrose	+	+
Urease	+	-
H_2S production	-	+

+= Positive; - = negative; F = fermentative; G = green; Y = yellow;

Table 2

Sensitivity of various bacteria isolated from swollen hindgut syndrome PL to different drugs

Antibiotics	*V. alginolyticus*	*V. harveyi*
Ampicillin (10 µg)	R	R
Amoxicillin (30 µg)	R	R
Chloramphenicol (30 µg)	S	R
Erythromycin (15 µg)	I	R
Ciprofloxacin (5 µg)	S	S
Furazolidon (20 µg)	R	I
Nitrofurazone (100 µg)	R	R
Norfloxacin (10 µg)	S	S
Metronidazol (20 µg)	R	R
Oxytetracyclin (30 µg)	I	S
Penicillin-G (10 U)	R	R
Co-trimoxazole (10 µg)	R	R
Streptomycin (10 µg)	R	R
Tetracycline (30 µg)	I	I

S=sensitive (18-25 mm or more), R=resistant (6-12 in mm), I=intermediate (13-17 in mm)

Conclusions. To prevent this problem (SHG) some technique can be adopted which includes improving water quality, adding digestive enzymes to the feed by top-dressing; using newly hatched *Artemia* nauplii; using enriched *Artemia* nauplii; reducing the use of antibiotics and MIC testing to select and use only permitted and effective antibiotics. Antibiotics will only work against bacteria that are sensitive to that antibiotic, so shrimp hatcheries must be check first whether the problem is bacterial and then culture the problematic pathogen(s) and conduct sensitivity analysis with legal antibiotics to test the effectiveness of antibiotics before use.

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