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Ulcer Type of Disease in the Fishes of Small-Scale Farmer's Pond in Bangladesh

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Abstract: Studies were conducted to investigate ulcer type of disease in the small-scale rural poor fish farmer's pond and to suggest a low-cost treatment measure. In total 20 ponds of 10 different sites were selected in the district of Mymensingh for treatment and control ones. Fishes were sampled on monthly basis starting from September 2000 and continued until April 2001 to examine their disease condition. Ulcer type of disease with expression of lesions was detected by spot observation followed by laboratory examination. A number of suspective bacterial pathogens were recovered from the lesions and kidney of diseased fishes, viz., *Cirrhinus cirrhosus, Labeo rohita, Catla catla, Barbodes gonionotus, Pangasius hypophthalamus.* Fungal isolates especially *Aphanomyces* sp., *Saprolegnia* sp. and *Achlya* sp. were recovered from the lesions and affected muscles of the sampled fishes. A number of recovered bacterial isolates were detected as pathogenic among which *Aeromonas hydrophila*, Ah-11 and *A. veronii* biover *sobria* were recognized as high virulent isolates. In the case of fungal isolates *Aphanomyces* sp. and *Saprolegnia* sp. were detected as pathogenic. Prevalence of disease outbreak was found to be very low where preventive treatment measures were taken with salt and lime (1:1, 1 kg/decimal). In the winter months (January and February), the disease outbreaks were found to be very high in the non-treated (control) ponds, whereas in the treated ponds prevalence of infection were significantly low. In laboratory based treatment trial, antibiotic renamycin was found to be effective against bacterial invasion at a dose of 50mg/kg body wt/day applying for five days. In the case of fungal infection, the diseased fishes were found to be cured within five days by one hour bath in 0.5% salt and lime suspension at a ratio of 1:1 applying for 3 days.

Key words: Small-scale farmer's pond, ulcer disease, pathogen, low-cost treatment, infection

Introduction

Disease has become one of the major limiting factors in aquaculture production of Bangladesh, especially with the recent increase of aquaculture practices in order to fulfill the protein deficiency of the rural people. Rural small-scale aquaculture is the extensive or semi-intensive low-cost farming of aquatic organisms by households or communities, using technology appropriate to their resource base (Edwards and Demaine, 1997). Common diseases of freshwater fishes are ulcers including epizootic ulcerative syndrome (EUS), septicemia disease, tail and fin rot, gill rot, dropsy, various types of fungal, parasitic and protozoan disease (Chowdhury, 1997). Ulcer types of diseases including EUS are often confusing for their expression and causative agents. These are found to occur through out the year in both farmed and wild fishes. Due to lack of proper knowledge of fish health management, farmers have to fail in most cases to control the disease and become helpless to save their fish stock. Many of the rural poor farmers are frequently used to come to the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh seeking suggestion to solve their disease problems. Information on rural based small-scale farmers pond regarding diseased status, their causative agents, suitable control measures etc. are very important to these farmers who contribute a lot to increase total fish production in the country. But research on this aspect has not yet been done systematically in the country. Considering the importance, the objective of the present study was to investigate ulcer type of diseases in the small-scale rural farmer's pond, their causes and to suggest suitable low-cost control measures.

Materials and Methods

Pond selection and sampling: In total 6-8 decimal sized 20 ponds belonging to rural poor farmers were selected in different sites in Mymensingh district including two ponds of an NGO (GRAMAUS) Among these, 10 ponds were selected for application of preventive treatment trial and 10 were without treatment which were individually situated beside treated ponds considered as the control. Fishes were sampled monthly basis for eight months starting from September 2000 and continued up to April 2001. In each sampling 50 fishes of different species were

caught by a cast net and grossly checked their disease condition with any abnormality or expression of lesions on which prevalence of disease outbreak was based. Among the diseased fishes, randomly selected fishes were sampled and immediately brought to the laboratory for isolation of suspective pathogenic bacteria and fungi.

Isolation and identification of bacterial pathogens: Bacterial swabs were taken aseptically from the lesions, kidney and liver of the diseased fishes and cultured on the selective media Aeromonas Agar Base (Oxoid) supplemented with ampicillin SR 136E, Pseudomonas Agar Base (Oxoid) supplemented with antibiotic C-F-C (Centrimide-Fucidin-Cephaloridine) SR103E and SS Agar. The first two selective media were found to be suitable for isolation and determination of aeromonads and pseudomonads, respectively (Chowdhury and Inglis, 1994a, 1994b). Pure cultures of *Aeromonas* spp., *Pseudomonas* spp. and *Edwardsiella* spp. were obtained from selective agar culture plate. The isolates were maintained in TSA slant at 4°C. Suspective bacterial pathogens were primarily identified up to genus level according to the flow chart diagram of Tonguthai *et al.* (1999). In order to identify bacterial isolates, API-20E test kit procedure was followed in addition to the conventional method described in Cowan and Steel's Mannual for the Identification of Medical Bacteria after Barrow and Feltham (1993). However, species of *Aeromonas* were confirmed after Carnahan *et al.* (1991).

Isolation and identification of causative fungi: Fish with pale, raised lesions, which was not completely ulcerated, was considered for fungal isolation. Different types of affected fish (wild and farmed) were sampled from Mymensingh area and immediately brought to the laboratory in the same water where sampled carefully for isolation. Affected muscles were aseptically collected with the help of a sterile scalpel and immediately placed in petridish containing isolation medium, GP-PenOx broth suggested by Willoughby and Roberts (1994). The fungus was transferred to fresh plates of GP-PenStrep agar until cultures were free of bacterial contamination. The fungus was then subcultured on GP-agar at intervals of no greater than 5 days. To induce sporulation, an agar plug (3-4 mm in diameter) was placed in a petridish containing GPY broth and incubated for four days at 20^0C. The nutrient agar out of the resulting mat was washed by sequential transfer through five petridish containing autoclaved pond water (APW) and kept overnight at 20^0C in APW. After about 12 hours the formation of primary cysts and release of motile secondary zoospores were observed under microscope. The genera *Aphanomyces, Saprolegnia, Achlya* were distinguished primarily by asexual characters, especially zoosporangial shape, method of zoospore release and method of zoosporangial renewal following the methods described by Willoughby (1994) and Lilley *et al.* (1998).

Pathogenicity study of recovered aeromonad and pseudomonad bacterial isolates: Healthy young *Puntius gonionotus* of 20 to 30g in weight maintained in the aquarium were used as experimental fish, which were checked for any disease before exposure to bacterial suspension.

The fish were injected intramuscularly with 0.1ml of bacterial suspension of the pre-fixed dose of 2.5×10^6 CFU/ml at the base of dorsal fin of the experimental fish. Control fish received only sterile physiological saline. Five fish were kept in 10 litre of tap water in a 15-litre capacity glass aquarium. Aeration was maintained in each aquarium and in every 24h 50% of water was exchanged with tap water. Water temperature was maintained with air temperature which did not vary the range of 22 - 24°C. No feed was given during the experimental period. Appearance of lesions, moribund and death were considered as primary diagnosis of infection. However, the infection was confirmed by re-isolation of the pathogens from kidney of the affected or moribund fish and its histopathological study. The experiment was continued up to 10 days.

Pathogenicity of recovered *Aphanomyces* and *Saprolegnia* fungal isolates: Experimental fish (*Puntius gonionotus* and *Pangasius hypophthalamus*) were separately challenged against the two types of fungal isolates. The fungal isolates *Aphanomyces sp.*, Ap-1 and *Saprolegnia sp.*, Sa-11 were separately cultured on GP agar at 22°C. Suspension of motile secondary zoospores was prepared in autoclave pond water. The fish were stressed by a scalpel abrasion on the trunk region and immediately exposed to fungal suspension adjusted to a dose of 1×10^4 spores/ml. Five fish were maintained in each aquarium. Aeration and change of water was maintained as described before. Control fish were treated as same without contacting with fungus. The experiment was conducted up to 10 days and infection was monitored observing fish behaviour, appearance of lesions or mortality of fish.

Chemotherapy under laboratory condition: Growth and multiplication of fungal culture were checked under *in vitro* condition using GP broth mixing with selected chemicals such as salt, lime etc. In order to find out suitable treatment measure against bacterial and fungal

disease in the fishes cultured in different ponds, chemotherapeutic treatment trial was performed on the diseased sample fish under laboratory condition. Disease affected 15 pangas from Bailer, 15 pangas from Fulpur and 40 sarputi from Bangladesh Agricultural University (BAU) fish farm were brought to the laboratory in alive condition and maintained them in separate aquaria with aeration. The fishes were bathed in the suspension of salt, combined salt and lime and antibiotic renamycin at their different doses. Renamycin was also used with feed.

Preventive treatment under field condition: A preventive treatment based on previous preliminary study was applied in the selected 10 ponds under the present study. In order to compare the effect of this treatment, nearby one pond against each treated pond was selected as the control giving no treatment. A dose of 1kg salt and 1 kg lime per decimal of water area was applied to each treatment pond at the beginning of the winter season (November). Applications of the second and third dose were given at two weeks interval where the doses were half of the initial. Outbreak of the disease was investigated throughout the eight months sampling period (September 2000 to April 2001).

Results

Bacterial and fungal isolates: In disease investigation, fishes were found to be affected by both bacteria and fungi. During December 2000 to February 2001 ulcer type of disease especially *Aphanomyces* infection associated with other was very common often producing typical EUS lesion in most of the investigated ponds. In total 44 bacterial isolates and 20 fungal isolates were recovered from several affected fish species of different treated and non-treated ponds through primary diagnostic procedure by which bacteria and fungi were identified up to genus level. Recovered bacterial isolates were categorised into 3 genera, viz., *Aeromonas, Pseudomonas and Edwardsiella* and in the case of fungal isolates also three genera were primarily identified as *Aphanomyces*, *Saprolegnia* and *Achlya* (Table 1). Among the total recovered bacterial isolates, numbers of Aeromonas were 27, *Pseudomonas* 9, *Edwardsiella* 8.

In the case of fungal isolates, numbers of *Aphanomyces* were 10, *Saprolegnia* were 7 and *Achlya* were 3. *Aeromonas* isolates were identified up to species level. These were *A. hydrophila* and *A. veronii* biovar *sobria*. Among the fungal isolates only *Aphanomyces invadans* could confirmed up to species level.

Pathogenicity test: Among the recovered isolates, a number of isolates were detected as pathogenic through experimental infection under laboratory condition. Representative of these bacterial and fungal isolates were found to invade the challenged fish successfully producing lesions or killing them (Table 2, 3).

The isolate, *Aeromonas hydrophila*, Ah-11 was found to be more pathogenic than those of *Aeromonas hydrophila*, Ah-2, *A. veronii* biover *sobria* As-9 and As-17 (Fig. 1). *Pseudomonas* isolates, *Pseudomonas* sp., P-2 was comparatively more pathogenic than *Pseudomonas* sp., P-5 (Fig. 2). In the case of fungal isolates, *Aphanomyces* sp., Ap-1 invaded the experimental fish produced lesions and caused mortality higher than those caused by *Saprolegnia* sp., Sa-11 (Fig. 3).

Response to different chemotherapeutants: In response to different chemotherapy treatment under laboratory condition, salt and lime combinedly was found to have suppressive effect on the growth of *Aphanomyces* in GP broth (Table 4). No effect of ash was detected in reducing the growth of the fungus. But salt, lime, $KMnO_4$ had moderate to weak effect in reducing the fungal growth under *in vitro* condition. The antibiotic, renamycin (Oxytetracycline) was found to have positive effect against the bacterial invasion at a dose of 50mg/kg body wt/day applying for five days. Diseased fish caused by fungus were found to recover 70-80 % by one hour bathing in 0.5% salt and lime suspension at a ratio 1:1 applying for 3 days. In the case of bacterial disease 80-90% affected fishes were found to recover when treated with renamycin (Table 5).

Chemotherapy under field condition: In field trial, a preventive treatment with salt and lime together was found to be effective to check the occurrence of disease. In most of the treated pond including two NGO's ponds no disease was detected during the investigated periods. Prevalence of disease outbreak was common in all of the control ponds (non-treated) which was peak in the month of January followed by February 2001 (10-32 %) (Fig. 4). During this period disease was also found to occur in the four treated ponds with less prevalence (2-6 %).

Discussion

The present study provides information that the fishes of small-scale farmer's pond have to suffer from ulcer type of disease. The disease was found in the month of November 2000 until April 2001, peaked in the months of January and February, 2001. During this peak time most diseased fishes were found to be affected by EUS. The syndrome appeared to be seasonal (Tonguthai, 1985) and may be associated with environmental stress especially low temperature during the winter season (Macintosh and

Table 1: List of bacterial and fungal isolates recovered from different diseased fish species

	Bacterial isolates			Fungal isolates		
Fish species	*Aeromonas* spp.	*Pseudomonas* spp.	*Edwardsiella* spp.	*Aphanomyces* spp.	*Saprolegnia* spp.	*Achlya* spp.
Cirrhinus cirrhosus	A-1, A-4,A-9, A-11, A-13, A-20,A-25, A-27	P-2, P-5, P-8	E-3	Ap-1, Ap-7, Ap-9	Sa-2	Ac-3
Labeo rohita	A-5, A-8,A-17, A-9, A-26	P-3, P-7	E-2	Ap-2	Sa-4	Ac-2
Catla catla	A-6, A-15, A-21, A-24	P-9	-	Ap-3, A-7	-	-
Barbodes gonionotus	A-2, A-10, A-14, A16,A-18, A-22	P-1, P-4, P-6	E-5	Ap-4, Ap-10, Ap-8	Sa-6	Ac-1
Pangasius hypophthalamus	A-3, A-7, A-12, A-23	- -	E-1, E-4, E-6, E-7,E-8	Ap-6 -	Sa-1, Sa-3, Sa-5, Sa-7	- -

Table 2: Summary of pathogenicity test on the experimental fish challenged with *Aeromonas* and *Pseudomonas* bacterial isolates

		No. of fish affected at different days after exposure														
	No. of fish	Day-1		Day-2		Day-3		Day-5		Day-7		Day-10		Lesion appeared	Mortality	Re-
Islolates	exposed	L	M	L	M	L	M	L	M	L	M	L	M	(%)	(%)	isolation
A-2	10	2	3	4	2	-	2	-	-	-	-	-	-	60	70	+
A-9	10	2	5	3	3	-	2	-	-	-	-	-	-	50	100	+
A-11	10	5	5	-	5	-	-	-	-	-	-	-	-	50	100	+
A-17	10	4	5	-	2	-	1	-	-	-	-	-	-	40	80	+
A-20	10	4	6	-	-	-	2	-	-	-	-	-	-	40	80	+
A-27	10	4	2	2	1	1	1	-	2	-	-	-	1	70	70	+
P-1	10	2	-	1	1	2	1	-	2	-	-	-	1	50	50	+
P-2	10	3	3	1	-	2	2	1	-	-	1	-	1	70	70	+
P-3	10	-	-	1	-	2	2	1	-	-	2	-	1	40	50	+
P-4	10	3	1	2	2	1	2	1	-	-	2	-	1	70	80	+
P-5	10	-	2	2	2	2	2	1	-	-	-	-	-	50	60	+
P-8	10	1	-	3	1	2	1	1	2	1	-	-	1	80	50	+

L: Lesion, M: Mortality, +: Detected, - : No lesion/mortality, A-2, A-11: *A. hydrophila*, A-9, A-17: *A. veronii* biovar *sobria*, A-20, A-27: *Aeromonas* sp., P : *Pseudomonas* sp., Water temperature maintained at 22-23^{0}C

Table 3: Summary of pathogenicity test on the experimental fish challenged with *Aphanomyces* and *Saprolegnia* fungal isolates

		No. of fish affected at different days after exposure														
	No. of fish	Day-1		Day-2		Day-3		Day-5		Day-7		Day-10		Lesion appeared	Mortality	Re-
Islolates	exposed	L	M	L	M	L	M	L	M	L	M	L	M	(%)	(%)	isolation
Ap-1	10	-	-	4	2	2	3	2	2	-	2	-	1	80	100	+
Control	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sa-11	10	2	1	3	2	1	1	1	1	-	2	-	1	70	80	+
Control	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

L: Lesion, M: Mortality, +: Detected, - : Not detected, Ap: *Aphanomyces invadans,* Sa: *Saprolegnia*

Table 4: Sensitivity of *Aphanomyces* fungus to different chemotherapeutants under *in vitro* condition

Treatments	Chemotherapeutants	Dose (mg/20ml GP- broth)	Growth of *Aphanomyces*
T1	$KMnO_4$	0.03	++
T2	Salt	0.50	+
T3	Lime	0.50	+
T4	Salt + Lime	0.50	-
T5	Ash	0.80	+++
T6	Control	None	+++

- : No growth, + : Poor growth, ++ : Moderate growth, +++ : Optimum growth, Ratio of salt and lime 1:1

Table 5: Result of the treatment trial on the diseased fish under laboratory condition

Fish species	Type of Infection	Chemothera-peutants	Bath/Feed	Dose	No. of fish treated	Percentage of recovery
P. hypophthalamus	Fungal	Salt	1 hr. for 3 days	2g/l	10	70
P. hypophthalamus	Fungal	Salt + lime	1 hr. for 3 days	2g/l	10	70
B. gonionotus	Fungal	Salt	1 hr. for 3 days	2.5g/l	10	80
B. gonionotus	Bacterial	Renamycin	5 days	50mg/l	10	80
B. gonionotus	Bacterial	Renamycin	5 days (feed)	50mg/kg body wt.	10	90

Ratio of salt and lime 1:1

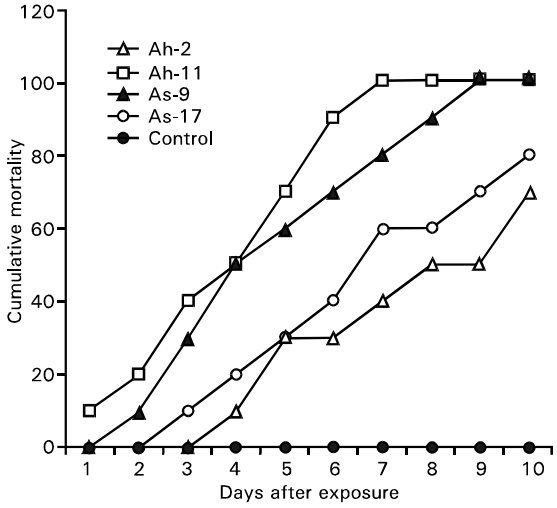

Fig. 1: Cumulative mortality of experimental fish at different days of exposure challenged with virulent *A.hydrophila* and *A. veronii* biovar *sobria*

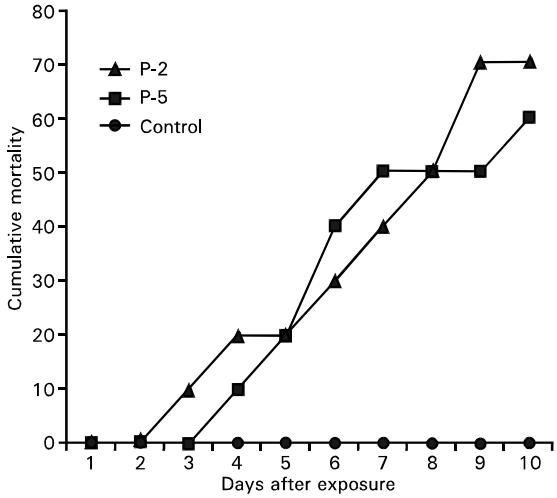

Fig. 2: Cumulative mortality of experimental fish at different days of exposure challenged with two virulent *Pseudomonas* spp.

Phillips, 1986). A number of studies shown low temperature delay the immune response in fish (Catap and Barry, 1998). Ahmed and Rab (1995) reported that *B. gonionotus* culture ponds were worst affected (64%) by EUS. Lilley *et al.* (1992) reported that Indian major carps were much more susceptible to EUS.

Among the recovered bacterial isolates, the dominant bacteria were *A. hydrophila*. and *A. veronii* biover *sobria.* The present finding support the previous works done by Sarker *et al.* (1999) and Roberts *et al.* (1989). Chowdhury

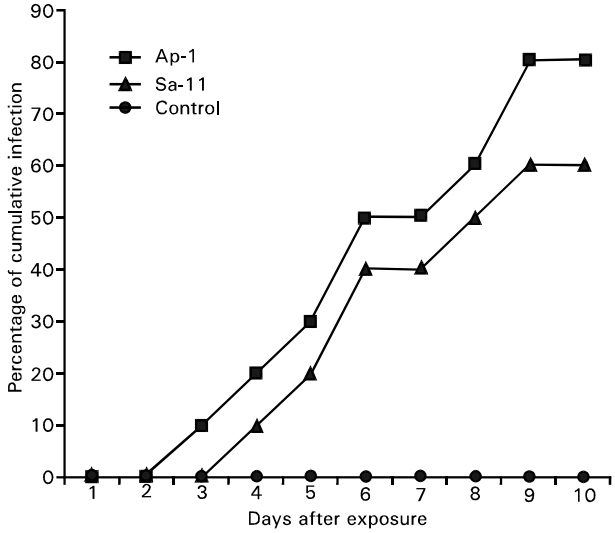

Fig. 3: Prevalence of cumulative infection of experimental fish at different days of exposure challenged with virulent *Aphanomyces invadans* and *Saprolegnia* sp.

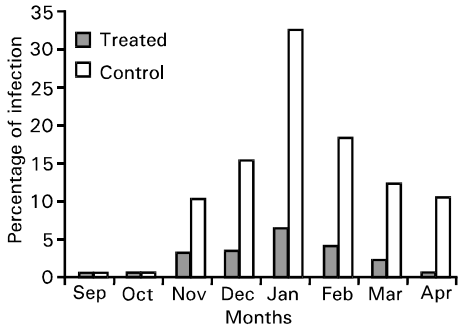

Fig. 4: Prevalence of disease outbreak in the fishes of treated and control ponds at different months

(1998) reported the involvement of aeromonads and pseudomonads in the ulcer type of disease in freshwater fishes. The involvement of *A. hydrophila*, *A. veronii* biover *sobria, Micrococcus* spp., *Flavobacterium* spp. and *Vibrio* spp. might be the cause of highly significant secondary infection agents in the EUS in fish (Lilley *et al.*, 1992). In this study, *Aphanomyces invadans* was detected as a very common fungal pathogen, especially in the diseased fishes sampled in the month of January and February 2001. However, *Saprolegnia* spp. and *Achlya* spp. were also the fungal pathogens recovered from the ulcer affected fishes. EUS was confirmed by the re-isolation of *A. invadans* and histopathological observation of mycotic granuloma in the affected fish

tissues. Among the investigated fishes *C. cirrhosus* and *B. gonionotus* were found to be severely affected by the disease. In the pathogenicity studies, all of the bacterial isolates tested were detected as pathogenic to the experimental fish by injection method where *A. hydrophila* was found to be more pathogenic than the other ones. The results directly correlate with the findings reported by Sarker *et al.* (1999). In the case of fungal pathogenicity test, although both *A. invadans* and *Saprolegnia* spp. were detected as pathogenic to fish but only *A. invadans* produced characteristic lesion which was very similar to the natural EUS lesion. The results support the findings obtained by Lilley and Roberts (1997).

In the study of chemotherapeutic trials, salt and lime were detected as effective chemotherapeutants against fungal infection when applied combinely. On the other hand, renamycin was effective against bacterial infection. However, the most interesting result was obtained when salt and lime were applied as preventive treatment with gradual and successive doses under field condition before starting of the disease. Both of these chemotherapeutants are cheap and locally available for small fish farmers and they can able to apply accordingly. Thus, it is suggested to apply salt and lime to prevent the severity of ulcer disease at eco-friendly low dose at successive interval. Further works are necessary to find out details of the infectivity of the pathogens and their control measures useful to poor small fish farmers.

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