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Research Article

PATHOGENESIS OF *FLAVOBACTERIUM COLUMNARE* IN FISH OF FRESH WATER RIVERINE ECOSYSTEM FROM EASTERN REGION OF UTTAR PRADESH, INDIA

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ABSTRACT

A bizarre form of bacterial necrosis in gills of tilapia, *Tilapia niloticus* and skin lesions in common crap, *Cyprinus carpio* were identified during the late summer season of the year 2015. *Flavobacterium columnare* was found to be responsible for pathogenesis in the selected fish of river Yamuna at Allahabad in the eastern region of Uttar Pradesh, India. It was a gram-negative, rod-shaped aerobic bacterium. The pattern of infection was very specific and sporadic to host and seasonality. The infection prevalence was recorded more in gills than body surface in both the fish during the period of investigation. All outbreaks occurred at optimum higher water temperature of summer season and it was positively influenced by pollution and medical discharge of neighboring area. The causative bacteria of this report were well documented for fish mortality and diseases to human beings. The present investigation dealt to monitor the pathogenic impact and their consequences for economic value point of view and its alarming causes to public health because investigated water body is related to religion faith.

INTRODUCTION

The description of the statement "microbiology" and "microorganism" is not an unproblematic mission (Farkas, 1985). The concern of aquatic animal welfare is in advance escalating Currency in the scientific and social community across the globe. The commencement and persistence of Bacterial gill disease (BGD) are accelerated by stress factors that upshot from rigorous culture. Although overcrowding, low dissolved oxygen concentrations, ammonia buildup, and manmade activities, as well as medicinal effluents, may contribute these diseases. However, when appropriate stressors are present, gill tissues are populated by filamentous yellow-pigmented bacteria that damage gill tissue and cause the death of the fish and sensitive aquatic fauna (Bullock, 1990; Malhotra, 2008). BGD was first reported by Davis (1926) but the causative agent was isolated on a bacteriological medium in 1978 (Kimura *et al*., 1978). The physiological and phenotypic characterization of the organism responsible for the gill disease was worked out from isolates recovered from fish of the United States, Japan, and Hungary (Wakabayashi *et al*., 1980; Farkas, 1985; Wakabayashi *et al*., 1989). The taxonomic and systematic nomenclature of the responsible bacterium as *Flavobacterium branchiophilum* was recognized and given after critical and proper analysis (Von Graevenitz, 1990). Infectious gill diseases in cultured and wild fish are commonly caused by yellow pigmented bacteria (Speare and Ferguson, 1989). Most of the yellow pigmented bacteria (YPB) have parallel cell morphology, but only a few produce distinctive branchial and skin lesions (Wakabayashi *et al*., 1980; Daoust and Ferguson, 1985). Infectious gill diseases in cultured and wild fish are commonly caused by yellow pigmented bacteria (Farkas, 1985; Speare and Ferguson, 1989). Most of the yellow pigmented bacteria (YPB) have parallel cell morphology, but only a few produce distinctive branchial and skin lesions (Wakabayashi *et al*., 1980; Daoust and Ferguson, 1985). For example, *F. columnare* give rise noticeable and disgustingly visible necrosis of the gill filaments, while *F. branchiophilum* produces a more disperse epithelial explosion in the nonexistence of unconcealed necrosis, foremost to branchial paleness and clubbing of the filaments (Toranzo, 2004). The Flavobacterial diseases cover a wide host range and spacious geographic allocation. This is due, in part, to their omnipresent nature and extensive assortment in water temperatures in which they persist (Swain *et al*., 2007; Starliper, 2012). The actual role of bacterial diseases to fish may vary from that of a primary pathogen to a stochastic opportunist invader which is in turn regulated or influenced by the pollution level of habitat, fish biology and behavior and cultural practices in selected areas described by various earlier workers (Davis, 1927;

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Toranzo, 2004). Freshwater cultured fish and fisheries are majorly devastated by bacterial diseases, including tilapia, catfish, carp, trout, salmon, bass, perch, eel and prawn (Upadhyay, 2016b). The infestation was optimum in warm water was proved by the reporting infection which was observed during the late summer season. Therefore, the causative agent was primarily expected might be *Flavobacterium, Staphylococcus or Aeromonas* because these species are known for their survival in warm fresh water. *F. columnare* was first described for disease columnaris or cottonmouth (Davis, 1927) as one of the oldest known diseases in warm water fish and fisheries from the Mississippi river (Declercq *et al*., 2013). The causative bacterium has been referred to by different names including *Bacillus columnaris, Flexibacter columnaris, Cytophaga columnaris* and most recently *Flavobacterium columnare* and reported from carp and Tilapia (Decostere *et al*., 2002; Mohamed and Saleh, 2010). Even though bacterial gill disease influences all age groups of fish but young and smaller fish are first and foremost affected. Disease outbreaks are frequent in spring and summer as water temperatures augment and when hatcheries classically have their utmost count of younger and smaller fish. BGD is widely considered to be a problem of cultured fish, particularly salmonids, and is also recognized as being problematic to wild fish populations in the present investigation. Unique clinical disease signs are associated with BGD (Morley and Lewis, 2010). Disease signs include lethargy, loss of appetite, gasping at the top of the water, and sluggish response to external stimuli such as hitting the side of a tank or waving a hand over the fish. The fish will swim high in the water column and align in a "soldier-like" fashion near and into the incoming tank water flow in an apparent effort to reach the coolest and most oxygenated water. These signs indicate that the fish are under high stress and in effort to respire. Mortality will quickly increase in the succeeding hours and days and if left untreated. The BGD in Indian freshwater fishes was caused by 14 other pathogenic bacteria including *F. columnare* investigated by earlier microbiologists (Dilip, 1985; Manna, 1998). The BGD and skin lesion in freshwater fish of lotic and lentic ecosystem was extensively worked out by the earlier workers in yesteryears but the present site of investigation has not been explored, therefore taken in consideration. Despite the prevalence and impact of BGD, however, very little epidemiological research has been conducted to describe outbreaks of this disease under field conditions, and identify and quantify important risk factors that influence the occurrence of BGD on fish farms. Because diseases of farmed fish typically have a multifactorial etiology, epidemiological information gained from studying risk factors for diseases of cultured fish is considered essential for the prevention and control of such outbreaks.

MATERIALS AND METHODS

The fish (tilapia, *Tilapia niloticus* and common carp, *Cyprinus carpio*) were collected from upstream to confluent point of trio holy rivers Ganga, Yamuna and Saraswati (Sangam in Hindi) as described in Vedas at Allahabad, Uttar Pradesh, India for isolation and identification of bacterial flora responsible to gill disease and necrosis or lesions in skin and their consequences in Indian fisheries. The site of investigation has very important fishing values and public health concern. Therefore, the inferences of a bacterial report from this selected site are alarming to pollution, climate change, geography and demography of fish and fisheries fauna. The live fish were collected from the Gau ghat, Allahabad, the eastern region of Uttar Pradesh, India during summer season (May-June, 2015). Fish were brought live to Laboratory, Department of Zoology, Ewing Christian College, Allahabad, Uttar Pradesh, India. The body surface and gills were observed carefully for necrosis and rotten symptoms. Under the positive observations, the infection site was swabbed and culture in nutrient medium. The infected gills and skin of fish were photographed subsequently. Catalase, oxidas, haemolysis, pathogenesis and culture-specific activities were critically worked out for the characterization of bacterial strain and pathogeneses (Desai and Desai, 1980). Samples were aseptically inoculated on nutrient agar (Himedia) to recovered pure culture (Toranzo, 2004; Malhotra, 2008). From a single colony, broth culture were created and 0.1ml of each isolate was serially diluted (10^{-1}-10^{-10}) after 24hrs of incubation at 36-38°C. The colonies obtained were counted by the standardized formula. The growth on nutrient agar and Ordal's medium (Robert *et al*., 1998) was very good biochemically it was a haemolysis positive (pathogenic) and gram-negative, aerobic, rod-shaped strain with CFU $3.24x10^5$/ml. The infections were characterized and compared and their correlation was worked out (Robert *et al*., 1998; Declercq *et al*., 2013).

RESULTS AND DISCUSSION

A sum of 77 fish (*T. niloticus*, 40 and *C. carpio*, 37) was examined during period of investigation (May-June, 2015). The collected fish were ranged 0.1-0.5 kg by weight and observed carefully for necrosis (Fig. 1) bacterial gill diseases (Fig. 2) and skin lesions (Fig. 3). The pattern of infection prevalence and disease symptoms were summarized in Table 1. There was 2.7% *C. carpio* with average body weight 0.325 kg, found to be infected by skin lesion in belly region immediate previous to pelvic fins. On contrary the *T. niloticus* were free from bacterial skin lesion or infection in belly region during the period of investigation. However, 17.5% Tilapia were observed with moderate to severe gill necrosis. The pattern and characteristics of observed infections were corroborated to the findings of earlier workers in analogous genera of the different geographical area (Robert *et al*., 1998; Declercq *et al*., 2013). The bacterial gill disease in India by *F. column*are was first of all investigated in Uttrakhand (Kumar *et al*., 1986) and later on in the eastern region of India (Dash, 2009). The findings of present investigation in the Gangetic plains were significantly corroborated with work of earlier investigators from different zoogeographic areas (Kumar *et al*., 1986; Dash, 2009). The causative agent of BGD, *F. branchiophilum*, is considered ubiquitous in the freshwater environment. When environmental conditions are favorable the pathogen is able to attach and proliferate on gill tissue, compromising gill function, and often leading to high mortality levels unless treatment is administered quickly and effectively. Environmental factors have long been recognized as being important in allowing *F. branchiophilum* to colonize gill tissue and cause clinical BGD and finding was corroborated by the present investigation.

Table 1 The observed bacterial infections in the fish of river Yamuna at Allahabad during summer season of 2015.

S. No.	Name of examined fish (Number of fish)	Fish infected by gill diseases (%)	Fish infected by skin lesions (%)	Total infection in fish (%)
1.	Common carp (37)	8.1	2.7	10.8
2.	Tilapia (40)	17.5	Nil	17.5
3.	Total fish (77)	25.6	2.7	28.3

Fig. 1 Bacterial gill disease (BGD) in fish tilapia, *T. niloticus*: (A) Gills with necrosis, (B) Magnified view of gill with yellowish necrosis, (C) Microphotographs of infected dermis of gills with necrosis (400X).

Fig. 2 Bacterial gill disease (BGD) in fish common carp, *C. carpio*: (A) Gills with necrosis as patches in epidermis and marginal infection, (B) Isolated gills with initial infection, (C) Microphotographs of infected gills with initial infection (100X).

Fig. 3 *In situ* lesion at belly before pelvic fin caused by bacterium in common carp, *C. carpio*.

The pathogenesis and molecular characterization of causative organisms in *Catla catla* was investigated from a natural fish pond in the sub-humid region of Uttar Pradesh, India (Verma and Rathore, 2013; 2015) and their symptoms, impact and pathogenesis was quite similar to the present investigation because of the same geographic area. The pattern of infection and occurrence in the selected area of investigation was influenced positively by the extrinsic factors, hydrobiological factors, and climatic factors. The bacterium *F. columnare* was found to responsible for the infection and columnaris in both, cultured and wild freshwater fish and fisheries including many commercially important aquatic fauna, investigated in the current investigation. *F. columnare* infections may result in skin lesions, fin erosion, and gill necrosis, with a high degree of mortality, leading to severe economic losses. Especially in the last decade, various research groups have performed studies aimed at elucidating the pathogenesis of columnaris disease, leading to significant progress in defining the complex interactions between the organism and its host (Declercq *et al*., 2013). The authors further explained that *F. columnare* can survive up to 16 days at 25°C in hard, alkaline water with a high organic load (Toranzo, 2004). The appearance of *F. columnare* infections and colonies characteristics of isolated bacterium from wounds were yellow, flat, thin, rhizoid in physical appearance and gram-negative, rod-shaped, aerobic biochemically and physiologically similar with symptoms discussed fish from warm polluted water during late summer season by yesteryear workers (Robert *et al*., 1998; Toranzo, 2004; Kumar, 2012). The symptoms of infection did not coincide between the genera of observed fish. The infections of *T. niloticus* were critically severe and diagnosed with gill necrosis while it was noticeable in the case of *C. carpio*. However, skin lesion was observed only in carp during the period of present study whereas, interestingly no skin lesion was experienced in tilapia (Julia *et al*., 2012). The distinguishing pattern of observed infection in both the fish species can be hypothesized on the basis of fish immunity, habitat characteristics and environmental condition of water body (Khan, 2012). The nature of infection in the freshwater fish was related to the level of cortisol during the reproductive period (Law *et al*., 2011). Therefore, these fauna are more prone to infection than the other period. The cortisol had weaker suppressive effects on the phagocytosis of leukocytes in common carp than tilapia, thereby, *C. carpio* reflected more leukocytes than *T. niloticus* under the phagocytosis activity in response to cortisol. Hence, directly it can be claimed that tilapia was immunologically weaker than common carp, in turn, the gills of respective fish were more infected than the other (Kumar *et al*., 2010). Comparatively more aggressiveness and residing behavior of *T. niloticus* under polluted water catalyzed and positively influenced above findings and mortality of fish during the extreme summer was also claimed by a few local fishermen during present investigation. Few outbreak of *F. columnare* were also reported in the confined water in yesteryear (Li *et al*., 2011; Verma and Rathore, 2013).

CONCLUSION

The observed infection was confined as *F. columnare* that was associated with warm water temperature and organic pollution in investigated water body. *F. columnare* has a wide range of host and worldwide occurrence. Due to the aggressive nature and survival under polluted water, only *T. niloticus* and *C. carpio* got an infection of *F. columnare*. The mortality and outbreak has also been reported by *F. columnare* but due to lotic nature and seasonal thermal stratification and oscillations of investigated water body, the infection was not observed serious in fish. But the presence of bacterial infection inside such an important water body is serious to public health and economy of local and National fishing that encourage its further data collection and specific characterization.

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