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HISTOPATHOLOGICAL CHANGES IN GILL OF *CLARIAS BATRACHUS* INDUCED BY EXPERIMENTAL *PROCAMALLANUS* INFECTION

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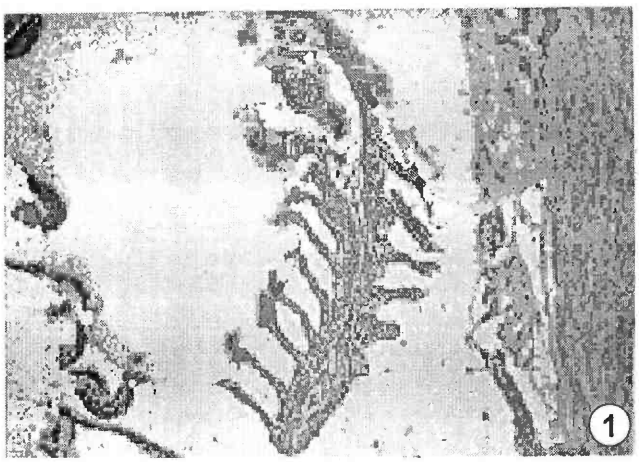
ABSTRACT:– **Alterations in gill of *Clarias batrachus* induced by experimental *Procamallanus* infection were recorded on day 15, 30, 45 and 60. Gill of the control catfish comprised laterally compressed leaf-like filaments (primary gill lamellae) arranged alternately on either side of the interbranchial septum. Each primary filament bore a row of secondary gill lamellae on both sides perpendicular to its long axis. Primary gill lamellae consisted of a central core of cartilaginous rod, lining epithelial cells and blood vessels whereas secondary lamellae comprised a layer of flattened epithelial cells attached to the basement membrane, contractile pillar (pilaster) cell system and blood spaces (sinusoid). Thyroid follicles with homogenous eosinophilic colloid material in the lumen were also seen scattered among gill lamellae. *Procamallanus* infection in the catfish induced mild hypertrophy as well as hyperplasia in the epithelium lining cells of the primary and secondary gill lamellae on day 15 and 30. Marked hyperplasia and telangiectasis were observed in secondary gill lamellae of the catfish due to *Procamallanus* infection by day 45 and 60.**

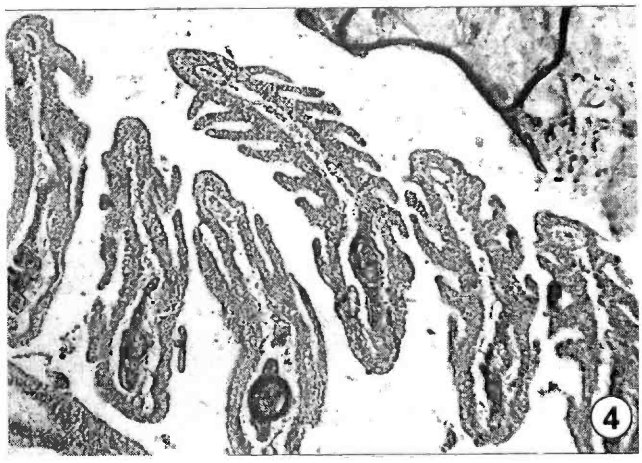
Key words : *Procamallanus* infection, gill, histopathology, *Clarias batrachus.*

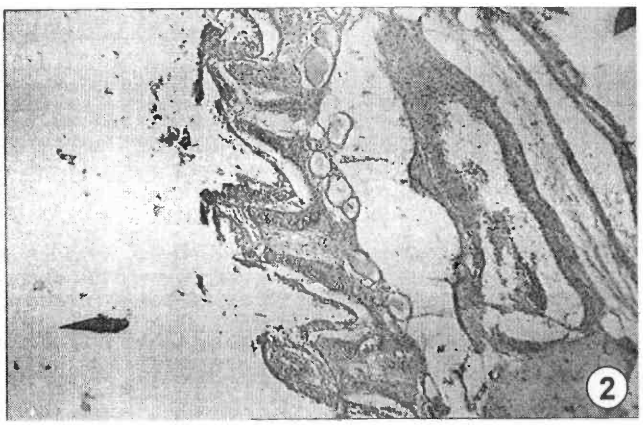
INTRODUCTION

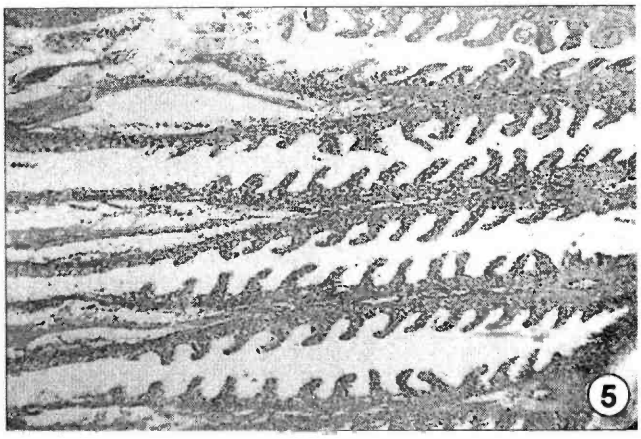
Metazoan parasitic diseases are most common in fishes and encounter more frequently than microbial infections in natural as well as culture systems (Madhavi, 2003). Sometimes mass fish mortality occurs specially in nursery as well as culture ponds and rivers. High stocking density, poor husbandry, occurrence of vectors, high organic load and unfavorable environmental temperature are also equally important contributing factors for parasitic diseases which induce various pathological changes in fishes (Roberts, 2001). Gastrointestinal helminths affect mankind as well as domesticated animals. The most costly parasites in terms of productions losses are gastrointestinal nematodes of ruminants and hogs (Gamble and Zarlenga, 1986). Histopathological manifestations such as marked lesions in the mucosa, inflammation and congestion of mucous glands and intestinal hemorrhages lead to severe anemic condition in the fish (Williams, 1967; Anderson *et al.*, 1976; Roberts, 2001). Besides mechanical injuries, atrophy of tissues and lesions of the alimentary canal, blood vessels or ducts etc, the parasites also introduce toxic metabolic byproducts (endotoxins/exotoxins) eliciting changes in the blood profiles, enzymes, vitamins and hormonal activity of the host (Poynter, 1966; Beckage, 1997; Mongkol-Primpol, 2000; Ekman and Norrgren, 2003; Kobayashi *et al*, 2004; Ruhela *et al*, 2006a, b).

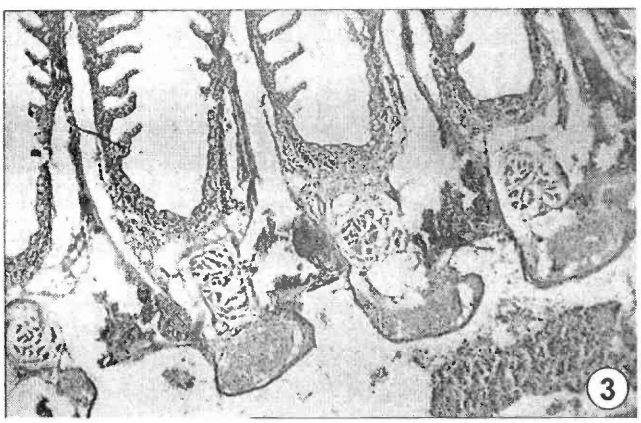
Procamallanus is a common nematode parasite in stomach and intestine of fishes inhabiting freshwater, brackishwater and marine ecosystems of the world (Yamaguti, 1961; Sood, 1988; Sinha, 1988; Zaman and Leong, 1988; Chandra, 1994; Martens and Moens, 1995; Bijukumar, 1996; Bharatha Lakshmi, 2000; Bharatha Lakshmi and Kumari 2000; Gonzalez-Solis *et al*, 2002; Moravec *et al*, 2004). Though the intermediate host (vector) of the parasite is copepods (Chandra and Modak, 1995; Sinha, 2000), there exist reports that both adult as well as larval stages of *Procamallanus* are pathogenic to fish (De and Maity, 2000; Ruhela *et al*, 2006a, b). Infestations of *Procamallanus* have been recorded in *Heteropneustes fossilis* and *Clarias batrachus* (Furtado and Low, 1973; Basirullah and Hafizuddin, 1974; Sinha, 1988; Zaman and Leong, 1988; Chandra, 1994; Katoch, 2002). Helminth infection causes deterioration in food value of the flesh and may result in heavy mortality of fishes (Madhavi, 2003). Further, heavy parasitic worm burden in the fish may reduce reproductive potential or delay sexual maturity serving as a limiting factor to population size of the species. Mongkol-Primpol (2000) reported the occurrence of *Procamallanus planolatus* in stomach and intestine of *Clarias macrocephalus* and observed cloudy swelling, fibrosis and degeneration in kidney of the infected fish. Though life-history, distribution and parasitic infestation of *Procamallanus* have been











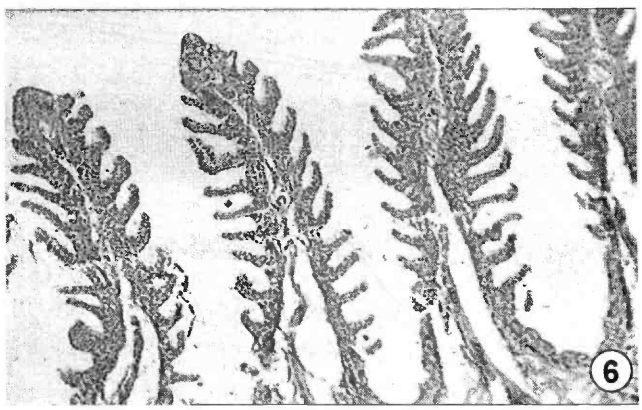


Fig. 1 : Gill of control *Clarias batrachus* showing primary and secondary branchial lamellae. H&E x 200.

Fig. 2 : Branchial region of catfish exhibiting distribution of thyroid follicles with varying amount of colloidal material (arrow). H&E x 200.

Fig. 3 : Gill of catfish on day 15 of *Procamallanus* infection showing mild hyperplasia at base of primary and secondary gill lamellae. H&E x 200.

Fig. 4 : Gill of catfish on day 30 of *Procamallanus* infection showing mild hyperplasia of epithelial lining cells of primary and secondary gill lamellae. H&E. x 200.

Fig. 5 : Gill of catfish on day 45 of *Procamallanus* infection depicting hyperplasia of epithelial lining cells of primary and secondary gill lamellae. H&E x 200.

Fig. 6 : Gill of catfish on day 60 of *Procamallanus* infection depicting hyperplasia of epithelial lining cells of primary and secondary gill lamellae. Mark the telangiectasis of secondary lamellae (arrow). H&E x 200.

described by several workers, histopathological alterations in tissues of the host due to toxic substances secreted/excreted by the parasites, particularly in the branchial tissues has not yet been studied. Therefore, an attempt was been made to record the alterations in gill of *C. batrachus* elicited by experimental *Procamallanus* infection.

MATERIALS AND METHODS

Clarias batrachus (Linnaeus) used in the present study were collected from local freshwater ponds and also purchased from fish markets of Meerut and adjoining region in Uttar Pradesh, India. Fishes were acclimatized to laboratory conditions for about a week before initiating the experiment. The adult female *Procamallanus* were collected from infected *C. batrachus* by cutting open the intestine. They were kept in watch glass filled with saline solution for natural egg laying at 24-27°C. The eggs were kept in Lock-Lewis solution for healthy embryonation. The solution was changed periodically and 0.1% formalin added to the culture medium to avoid fungal contamination of the eggs. 40 healthy catfish were randomly selected and divided into two equal groups. Catfishes of group 1 were not given any treatment and served as control whereas in catfish of group 2 experimental infection was induced by forcefully pushing 500 embryonated eggs of the nematode into the stomach of each catfish by means of a long-nozzled dropper (De and Maity, 2000; Ruhela *et al*, 2006a, b). Catfishes from the experimental as well as control groups were killed on day 15, 30, 45 and 60 and gills were surgically removed and fixed immediately in freshly prepared aqueous Bouin's solution. After routine processing in ascending series of alcohol and clearing in xylene, the tissue were embedded in paraffin wax at 60°C. Serial sections were cut at 6 µm and stained in hematoxylin and eosin for histopathological examinations.

RESULTS AND DISCUSSION

Gill of the control *C. batrachus* comprised laterally compressed leaf-like filaments (primary gill lamellae) arranged alternately on either side of the interbranchial septum. Each primary filament bore a row of secondary gill lamellae on both sides perpendicular to its long axis. Primary gill lamellae consisted of a central core of cartilaginous rod, lining epithelial cells and blood vessels whereas secondary lamellae comprised a layer of flattened epithelial cells attached to the basement membrane, contractile pillar (pilaster) cell system and blood spaces (sinusoid) (Fig. 1). Thyroid follicles with varying amount of eosinophilic colloid material in the lumen were also seen scattered among the gill lamellae (Fig. 2). *Procamallanus* infection in the catfish induced mild hypertrophy and hyperplasia in the epithelium lining cells of the primary and secondary gill lamellae on day 15 and 30 (Fig. 3, 4). Marked hyperplasia as well as telangiectasis were observed in the secondary gill lamellae of the catfish due to *Procamallanus* infection on day 45 and 60 (Fig. 5, 6).

Gills cover more than 60% surface area of the fish and their external location renders them the most vulnerable target organ for pollutants as well as parasitic infection (Pandey, 1994; Pandey *et al*, 1996, 1997a, b; Roberts, 2001). The lamellar epithelium is the barrier between the blood and surrounding water and any damage to this affects not only ventilation but also ion exchange, secretory and excretory function of gills (Roberts, 2001). Parasites secrete/excrete certain endotoxins/exotoxins in circulation of the host which affect the other body tissues like intestine (Ruhela *et al*, 2006c), liver (Ruhela *et al*, 2007) and kidney (Mongkol-Primpol, 2000; Roberts, 2001; Ruhela *et al*, 2008) besides alterations in the blood constituents, enzymes, vitamins and hormonal activity (Poynter, 1966; Beckage, 1997; Mongkol-Primpol, 2000; Roberts, 2001; Ekman and Norrgren, 2003; Ruhela *et al*, 2006a, b). Though severe histopathological damages have been observed in intestine, liver and kidney of *Clarias batrachus* due to prolonged experimental *Procamallanus* infection (Ruhela *et al*, 2006c, 2007, 2008), the alterations in cytoarchitecture of gill of the catfish under similar treatment have been comparatively mild (Fig. 3-6) which may probably be due to the intimate contact of the organ with surrounding water leading to diffusion/leakage/transport of the endotoxins/exotoxins (Roberts, 2001).

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