

First report of massive renomegaly in adult striped murrel *Channa striata* (Bloch, 1793)

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Abstract

Channa striata, commonly known as striped murrel, is an emerging species in Indian aquaculture having high consumer preference and market value. In the present study, six numbers of *C. striata* (500±100 g) reared in two different cemented tanks were noticed with growth of heavy tumorous mass beneath bent vertebrae at the lateral side of the body cavity. Surgical excision revealed the presence of massive renomegaly, while, other vital organs appeared normal. Bacteriological and parasitological analyses revealed absence of any pathogen involvement in the tumorous mass formation. Further, histopathological analysis of renal tissue revealed compressed tubules, degenerative to necrotic changes in the tubular epithelial cells leading to sloughing of lumen, and infiltration of lymphoid cells and necrotic mass in the interstitium.

Introduction

Tumours and neoplasia are largely being studied in higher vertebrates, mammals and birds whereas, very little attention has been given to lower vertebrates especially to aquatic animals. However, subsequent findings have shown that the neoplastic process is ubiquitous and tumours in cold-blooded animals are identical to those in higher vertebrates (Schlumberger and Lucke, 1948). Among cold-blooded animals, fishes are the most numerous group in which various types of tumorous formation have been observed in different organs. Commonly found tumours in fishes are classified as tumours of mesenchymal tissues, epithelial tissues, nervous tissues, pigment cell tumours and hamartomas. The development of tumour in fish is multifactorial (Sahoo *et al.*, 2017). Tumour growth can occur in various tissues, but in fish, the skin, gills and other internal organs are particularly prone to tumour development due to frequent exposure to different environmental contaminants (Roberts, 2012).

There is limited scattered information available regarding renal tumour formation

in fishes. In higher vertebrates predominantly two types of neoplasia are observed, i.e. nephroblastomas and certain variant of adenocarcinomas. A similar trend has been observed in fish, with a notable distinction being the presence of more cases of primary renal lymphoma (Lombardini *et al.*, 2014). A typical case of renomegaly has been observed in a population of crucian carp with the presence of both polycystic conditions as well as adenomas. Further, the polycystic kidneys are described as a precursor to adenoma. The presence of myxozoan *Sphaerospora dykovi* was positively correlated with renal epithelial and hematopoietic cell hyperplasia (Hoole *et al.*, 2001). Another typical case of renomegaly observed in the lumpfish *Cyclopterus lumpus* L., was caused by an intranuclear microsporidian *Nucleospora cyclopteri* n. sp. (Freeman *et al.*, 2013).

Murrels are a group of mostly carnivorous fishes known for their ability to tolerate low levels of dissolved oxygen owing to the presence of accessory respiratory organ. They are also known to thrive in relatively poor water quality conditions and are often cultured in muddy waters

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(Kumar *et al.*, 2011). Recently, advancements in breeding technology and successful domestication of *Channa striata* have led to a significant increase in consumer preference for this particular species (Kumar *et al.*, 2018; 2021). Global aquaculture production of *C. striata* has increased from 480 t in 1950 to 17,847 t in 2014 (FAO, 2017). Due to the intensification of culture practices, several diseases have also been reported very recently from *C. striata*. As the intensive culture of this species is relatively new to this country, very few disease conditions are being reported in this species. Co-infection with different parasites and bacteria (Paul *et al.*, 2020), infection with *Acinetobacter baumannii* (Rauta *et al.*, 2011), *Aeromonas hydrophila* (Mohanty *et al.*, 2012) and epizootic ulcerative syndrome (Tonguthai, 1985) have been described in this species. The present investigation describes renomegaly in six adult broodstock of *C. striata*, detailing histopathological changes and investigating the involvement of bacterial or parasitic pathogens.

Materials and methods

Disease occurrence and clinical signs

Six brooders of *C. striata* (500±100 g) reared in two cemented tanks (each 15 m²) at a stocking density of 0.8 kg m⁻² in the farm of the ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, India, exhibited swollen ventral parts and bent vertebrae (attributed to tumour growth beneath the vertebrae) during routine sampling. The fishes exhibited clinical signs of sluggish movement, surfacing, reduced feed intake, delayed response to feeding and frequent isolation near the corners of the tanks. The fishes were collected and brought to the laboratory for further examinations. The water quality parameters recorded in the tanks at the time of sampling were: temperature 25°C; pH 7.6; alkalinity 135 mg l⁻¹ as CaCO₃ and dissolved oxygen 5.0 mg l⁻¹. The fishes were too weak and their gonadal development was severely affected; however, no mortality was noticed. Two fishes were randomly selected, euthanised with a high dose of MS222 (150 mg l⁻¹) and necropsied (Fig. 1).

The fishes were devoid of any skin lesions and visibly no pathological changes were noticed in any organs, except in the posterior kidney. Gross examination revealed a unilateral lobulated red coloured swollen homogenous mass (Fig. 2). The peritoneal cavity was devoid of any fluid.



Fig. 1. Lobulated renal tumorous growth in *C. striata* on necropsy



Fig. 2. Excised tumour showing poorly vascularised homogenous mass extending from the anterior part of posterior kidney

Histopathology, bacteriology and parasitology

Aseptically collected samples from liver and kidney were inoculated into tryptone soy broth (TSB) for bacteriological examination. Impression smears were also prepared to check any parasitic presence in the tumorous mass. The excised tissue samples (tumorous mass) along with other organs (liver and spleen) were placed in 10% neutral buffered formalin and processed for histopathology, further embedded in paraffin wax, sectioned and stained with routine haematoxylin and eosin (H&E). To confirm the involvement of any bacterial and parasitic pathogens in the formation of the tumorous mass, the tissue sections were also stained with Giemsa and Ziehl-Neelsen (ZN) acid-fast staining. The stained sections were analysed under a microscope (Zeiss Stemi Scope A1, Germany) and photomicrographed. The tumour tissue sample preserved in 100% ethanol was also subjected to DNA extraction (Sambrook and Russell, 2001) followed by 16S rRNA gene and 18S SSU rDNA gene amplifications using universal primers 16S F/R as well as ERIB1 and ERIB10, respectively, for detection of any imprints of bacteria or parasite (Fiala, 2006; Paul *et al.*, 2020). Myxosporean specific nested PCR was also carried out using the primer sets H2-H9 and Genmyxo3-H2 as described by Hanson *et al.* (2001) and Griffin *et al.* (2008), respectively. A 25 µL of PCR reaction mixture for all three reactions were made as per Paul *et al.* (2020). The reaction mixture was cycled on a Veriti thermal cycler (Applied Biosystems, USA) and the reaction conditions are given in Table 1. All the amplified PCR products were run on 1% agarose gel, stained with 10 mg ml⁻¹ ethidium bromide stain (MP Biomedicals, India) to confirm the presence of DNA product using standard molecular weight marker of 100 bp DNA ladder (Thermo Scientific, India). The gel was then photographed in a gel documentation unit (BioRad, Germany).

Results

No growth was obtained in TSB after 48 h of incubation and no parasitic imprints were noticed in the impression smear of

tumorous mass under the microscope. Further, there were no appreciable macroscopic changes in vital organs except the posterior kidney. Gross examination revealed a unilateral lobulated red coloured swollen homogenous mass of 21 cm² (3.5 × 6 cm). On histopathology, the renal tubules were found to be compressed and epithelial cells of the tubules showed degenerative to necrotic changes inside the tumorous mass. Whereas, the tubules in the non-tumorous mass exhibited extensive necrotic changes of the tubular epithelial cells, sloughing into the lumen and a lack of haematopoietic tissue (Fig. 3). Tumour mass showed massive infiltration of lymphoid cells, necrotic mass and compressed tubules (Fig. 4). No appreciable histopathological changes were observed in liver and spleen tissues. Further, no bacterial colonies were visible upon staining of the sections with Giemsa or acid-fast stain (Fig. 5). Giemsa staining of the histological sections also indicated absence of any parasite in the tumorous mass. Universal 16s/18s rRNA PCR and myxosporidean specific nested PCR did not produce any amplicon. This further confirmed the absence of any bacterial or myxosporidean parasites in the formation of the tumorous mass.

Discussion

Renomegaly is the abnormal and uncontrolled growth of renal tissue under the influence of biotic or abiotic factors. In the present study,

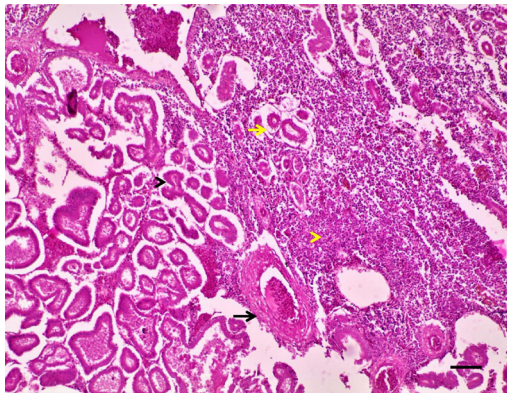


Fig. 3. Histopathology of tumorous mass. Left side i.e. non-tumorous part showing enlarged/distended tubules with disintegration of epithelial lining and sloughing into lumen (black arrow head) and congested blood vessels (black arrow). Right side i.e. tumorous part showing tumorous mass with presence of tubules. Detachment of tubular lining epithelial cells from the stroma (yellow arrow) and massive infiltration of lymphoid cells in the interstitium (yellow arrow head) (H&E). Scale bar represents 10 µm.

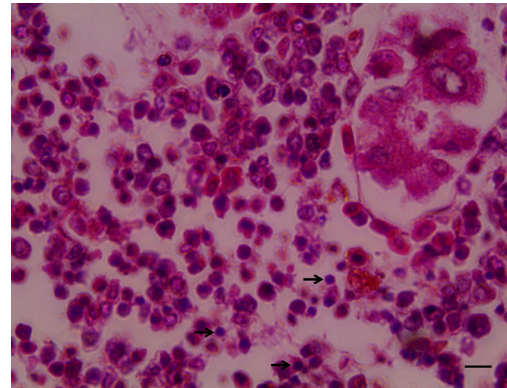


Fig. 4. Renal interstitium filled with dense round lymphoid cells of irregular sizes (H&E). Scale bar represents 25 µm

brooders of *C. striata* in a cement system exhibited abnormal growth beneath the vertebrae on the lateral side of the body cavity, leading to sluggish movement, surfacing, anorexia and cornering. Renomegaly was associated with significant clinical symptoms and extensive histopathological changes.

Granulomatous inflammation caused by *Mycobacterium* spp. (Hughes *et al.*, 2002) and microsporideans (Freeman *et al.*, 2013) have been reported from renomegaly cases of fish mortalities. In addition to infectious agents, several environmental factors such as pollution, carcinogens present in the water, irritants and mechanical injury can act as predisposing factors for tumour formation in teleosts (Lopez and Raibaut, 1981; Constantino *et al.*, 1999; Shokrpoor *et al.*, 2016). The tumorous growth found in the study was subjected to different bacteriological and parasitic investigations using level I, II and III diagnostics. The results of these tests were found to be negative, which implies non-involvement of any infectious agent in the formation of this particular renomegaly condition. Hence, the formation of renomegaly in this case may be of non-infectious nature. The other fishes stocked in the tank were found to be healthy without any clinical symptoms, further supporting the non-infectious origin of the tumour. The tumorous growth may be outcome of environmental carcinogens present in the water, which warrants further in-depth characterisation.

This is the first report of such tumorous mass formation in striped murrel, *C. striata*. The histopathological observations revealed several irreversible changes in the tumorous mass. Freeman *et al.* (2013) reported the formation of renomegaly and its associated histopathological changes in kidney tubules and haematopoietic

Table 1. Details of primers and PCR reaction conditions used in the study

Primer	Nucleotide base sequence (5'-3')	Product size (bp)	PCR conditions
16S-F	AGAGTTTGATCATGGCTCAG	1500	Initial denaturation at 95°C for 3 min; 45 cycles of: 30 s at 95°C, 1 min at 47°C, 1 min at 72°C and a final extension step of 10 min at 72°C
16S-R	GGTACCTTGTACGACTT		
ERB1	ACCTGGTTGATCCTGCCAG	1800	Initial denaturation at 95°C for 10 min; 30 cycles of: 1 min at 95°C, 1 min at 48°C, 2 min at 72°C and a final extension step of 10 min at 72°C
ERB10	CCTCCGCAGGTTCACTACGG		
H2	TTACCTGGTCCGGACATCAA	650	Initial denaturation at 95°C for 10 min; 30 cycles of: 1 min at 95°C, 1 min at 52°C, 2 min at 72°C and a final extension step of 10 min at 72°C
H9	CGACTTTTACTTCTCGAAATTGC		
Genmyxo3	TGATTAAGAGGAGCGGTTGG	900	
H9	CGACTTTTACTTCTCGAAATTGC		

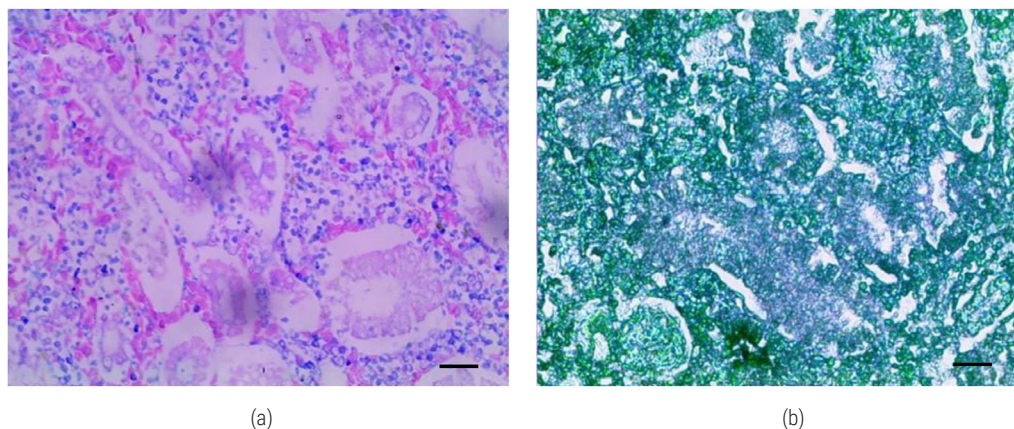


Fig. 5. Tumour section stained with (a) Giemsa and (b) ZN acid-fast stain. Scale bar represents 25 µm

tissue. The authors reported severe degenerative to necrotic changes in the renal structure. Further, a severe inflammatory reaction subsequently led to prominent hyperplasia in the renomegaly mass. Mullins *et al.* (1994) similarly reported infiltration of lymphocyte like cells in the kidney and other vital organs in response to microsporidian infection. Similarly, in this study tumours were found to be having massive infiltration of lymphoid cells, necrotic tissue mass and compressed tubules. The tumour formed was only observed in the anterior part of the posterior kidney and other vital organs were found to be normal. In addition, there were no appreciable histopathological changes observed in liver and spleen tissues which further confirms non-contagiousness of the tumorous growth observed in the kidney. At present, the formation of renomegaly, in this case remains speculative and further studies are required to determine exact aetiology. Given the significance of *C. striata* as a potential aquaculture species, these kinds of abnormalities should be given due priority.

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