

A Histological Study of Polypoidal Stomach in *Nemipterus japonicus* off Visakhapatnam Coast

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Abstract

Background/Objectives: Histopathological screening of a typical polypoidal stomach and associated organs was performed on *Nemipterus japonicus* off Visakhapatnam coast. **Methods:** 1832 specimens were collected from Visakhapatnam coast at a depth of 45m-100m within the period January 2011 to December 2013 were examined for documenting lesions in the stomach. Of the total samples about 18% of fish were identified with typical mucosal polyps which were autopsied and tissues of skin, gill, muscle, stomach, intestine, liver, spleen, kidney and gonads of both normal and diseased fish were fixed in 10% buffered formalin for 48 hours and dehydrated in graded alcohols, followed by impregnation in paraffin. The embedded blocks are cut 5 μ thick on a microtome and sections stained with haematoxylin and eosin, for the examination of pathological changes in the tissues. **Findings:** These studies revealed macroscopic lesions with a polyp arising from the mucosa of the gastric body leading to herniation of other digestive glands such as liver and pyloric caecum into the lumen of the polypoidal stomach. Histologically, the growth was differentiated as adenomatous polyp with abnormal proliferation of gastric mucosal glands in the stomach with focal erosion and hemorrhage. This evidence suggested chronic gastritis, but the cause of these unusual gastric polyps is unclear. Though earlier microbiological studies revealed the presence of enteric bacteria, with a dominance of *Klebsiella pneumoniae*, virological investigations failed to confirm any oncoviral particles and even aflatoxin analysis gave negative results. Immunological and karyological studies of *N. japonicus* with mucosal buccal polyps showed mitodepression. **Application/Improvements:** This is first histopathological report of this kind in *N. japonicus* and may help as a model for future studies on gastric polyposis in both human and animals.

Keywords: Adenomatous Polyp, Gastrointestinal Tract, Histopathology, *Nemipterus japonicus*, Visakhapatnam

1. Introduction

Fish health plays a key role in monitoring, evaluating and protecting aquatic animals within the ecosystem, in restoring the depleted populations or recovery of threatened and endangered species. Nemipterids are bottom dwellers frequently encountered with physical abnormalities and diseases¹ which appear to be associated with their environments². Incidentally typical gastric polyps have been manifested in *Nemipterus japonicus* off Visakhapatnam coast³. These are also supported by hydro toxic, karyological and immunological studies^{2,4,5}. Tumours have long been reported in different organs of marine fish. Visceral granuloma in brook trout, *Salvelinus fontinalis*⁶, dermal fibroma in a red band parrotfish, *Sparisoma aurofrenatum*⁷, systemic granuloma

in goldfish, neurofibromas in bicolor damsel fish *Pomacentrus partitus*⁸, skin tumors in Walleye⁹, visceral granulomatosis in wild atlantic cod *Gadus morhua*¹⁰, and in farmed large yellow croaker *Larimichthys crocea*¹¹.

There are many reports on chemically induced neoplasms such as hepatocarcinogenesis in *Cyprinodon variegatus*¹², and *Rivulusocellatus marmoratus*¹³, rainbow trout¹⁴, and *Oryzias latipes*^{15,16}. In spite of the above observations only few spontaneous gastric adenomas were reported in marine fishes such as intracardial mesotheliomas and a gastric papilloma in *Epinephelus itajara*¹⁷, tumors of the gastrointestinal system in the rainbow trout¹⁸ and medaka *Oryzias latipes*¹⁹ gastric polyp in a wild caught blue shark *Prionace glauca*²⁰. The present study on a gastric polyp in *Nemipterus japonicus* is the first of its kind. Since histological assessment in fish has

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been recognized as a highly valuable tool to identify the true health condition, this study is intended to perform a detailed histopathological analysis of different organs of these fish.

2. Materials and Methods

The specimens were collected regularly from research vessels of Fishery Survey of India, and also from local trawlers at a depth of 45m-100m within the period January 2011 to December 2013 off Visakhapatnam coast, 1832 fish were examined for documenting lesions in the stomach. Of the total sample about 18% of fish were identified with typical mucosal polyps which were subjected to autopsy. The entire digestive system along with mass in the mouth cavity is opened, washed gently with water to avoid ingested food material. Tissues of skin, gill, muscle, stomach, intestine, liver, spleen, kidney and gonads of both normal and diseased fish were fixed in 10% buffered formalin for 48 hours and dehydrated in graded alcohols, followed by impregnation in paraffin. The embedded blocks are cut 5 μ thick on a microtome and sections stained with haematoxylin and eosin, for the examination of pathological changes in the tissues²¹.

3. Results

At present we report a rare case of polypoidal lesion of size 1-2x4 cm in the buccal cavity of *Nemipterus japonicus* suggestive of neoplastic etiology (Figure 1). Polyp prevalence in Nemipterid population was about 18%. Affected fish were clinically normal except for those with large lesions in the gastric body, and significant weight loss caused by physical obstruction of food intake due to herniation of gastric stomach, completely enclosing the oesophageal cavity (Figure 2). In the present study, a wide spectrum of histopathological alterations were revealed in the skin, gills, muscle, stomach, intestine, spleen, liver, kidney and gonads of diseased fish. On aggregate, the stomach and intestine of fish accumulated more lesions and alterations.



Figure 1. Polypoidal mass in the buccal cavity.



Figure 2. Gastric stomach herniated into buccal cavity.

3.1 Polypoidal Stomach and Intestine

The normal mucosa of stomach was found to have numerous deep longitudinal folds lined by a few layers of stratified squamous epithelium with numerous superficial mucous cells (Figure 3). In abnormal cases, the gastric mucosa is highly folded and irregular in contour (Figure 4). There are multifocal nodular proliferations of glandular epithelium with folding and entrapment

of gastric crypts (Figure 5). Glandular cells form large lobules supported by dense fibrous stroma (Figure 6). In multifocal areas there is invasion of the submucosa by epithelial cells in large clusters or individuals as small acini associated with a dense schirrous stroma (Figures 7, 8). Granulomas containing numerous bacterial colonies are scattered within the mucosal and submucosal tissues.

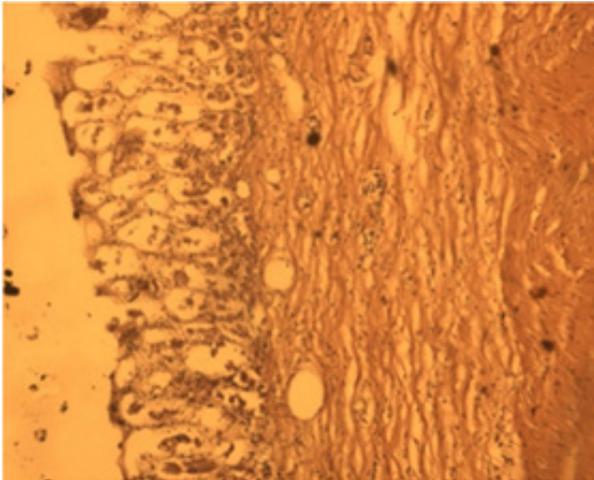


Figure 3. L.S of normal stomach (H&E x10).

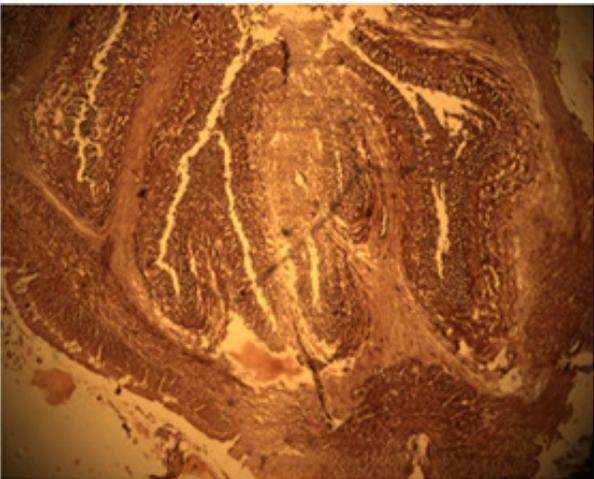


Figure 4. L.S of polypoidal stomach (H&E x10).

The intestine usually had a normal lining of simple mucoid columnar epithelium, which is visible as long folds with submucosa overlying the folds having eosinophilic granule cells, and is limited by dense mucosa and fibroblastic layer. In infected fish, the intestine revealed, catarrhal enteritis, with necrosis of enterocytes and intra luminal aggregation of mucous exudates, mucosal edema with erosion of glands along with congestion of blood vessels.

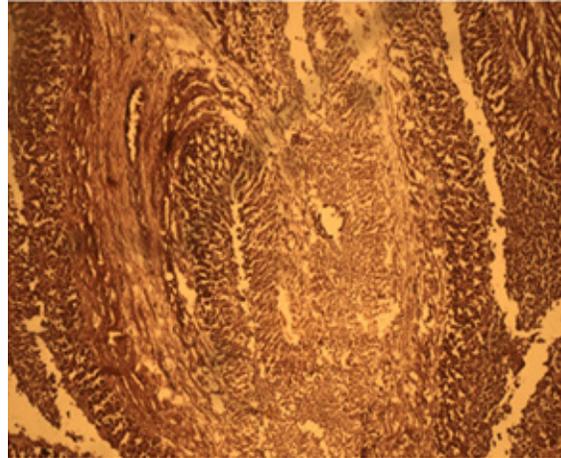


Figure 5. Junction showing inversion of mucosa (H&E x10).

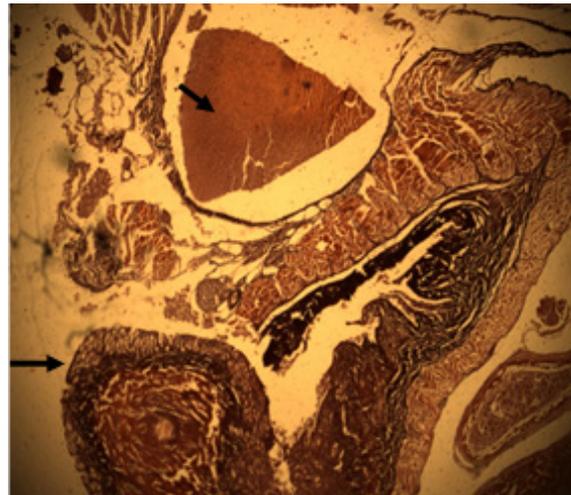


Figure 6. Mucosal prolapse and atrophy along with herniated liver and pyloric caecum enclosed in the lumen (H&E x10).

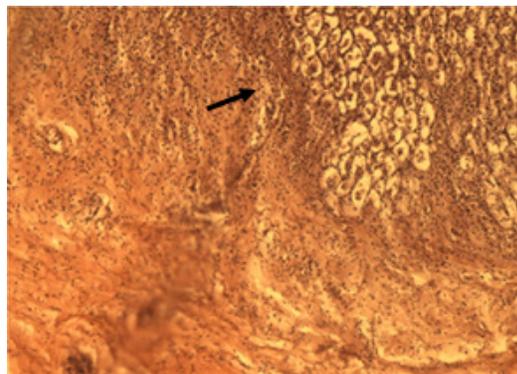


Figure 7. Necrotic mucosa with sub mucosal edema (H&E x10).

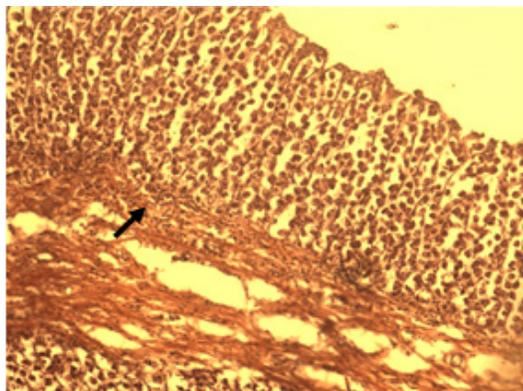


Figure 8. Fibrosis of lamina propria (H&E x20).

3.2 Skin, Gills and Muscle

Epidermis was constituted by an outermost layer of stratified epithelium and a basement layer of larger, undifferentiated cells, attached to the epithelium basal membrane. Dermis was formed mostly by connective tissue and includes the scale pockets. No external lesions were present in the skin of infected fish, and skin structure was consistent with normal teleost integument architecture. Compared with the above normal skin histology, the ulcerative skin showed loss of epidermis with thickening of the upper dermis. The underlying fascia and muscle were normal.

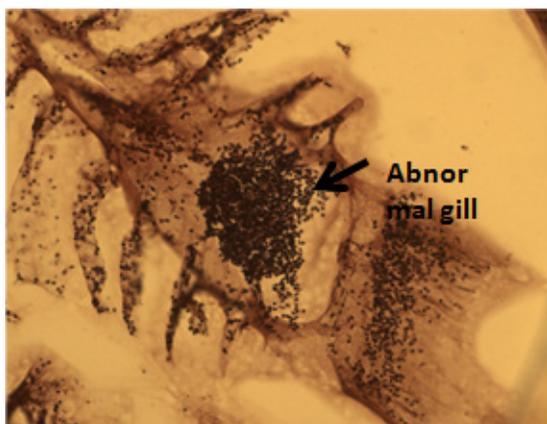


Figure 9. Section of gill showing proliferated inflammatory cells with congested lamellae (H&E x 10).

In the present study, gill damage was variable, however more pronounced in highly infected fish. Lesions and alterations in gills included low-moderate hyperplasia of interlamellar space epithelia, desquamation of lamellar epithelia and proliferated inflammatory cells with congested lamellae (Figure 9). Histologically, the normal

muscle bundles are very closely packed separated by thin tissue showing actin and myosin fibres for contraction and relaxation. The muscle fibres in the infected fish are of variable size showing focal loss of striations and loosely arranged fibrous connective tissue with lymphocyte proliferation.

3.3 Liver, Spleen, Kidney and Gonads

The liver showed vacuolar degeneration of hepatocytes, congestion of sinusoidal and hepatoportal blood vessels (Figure 10). The infected spleen revealed degraded RBC, disintegrated nucleus and necrotic tissue (Figure 11). In normal fish, the kidney has a large number of nephrons each having a renal corpuscle with a glomerulus and a capsule. In diseased condition, kidney lesions consisted mostly of necrosis and vacuolation of epithelial cells, lymphocyte proliferation with inflammatory changes (Figure 12).

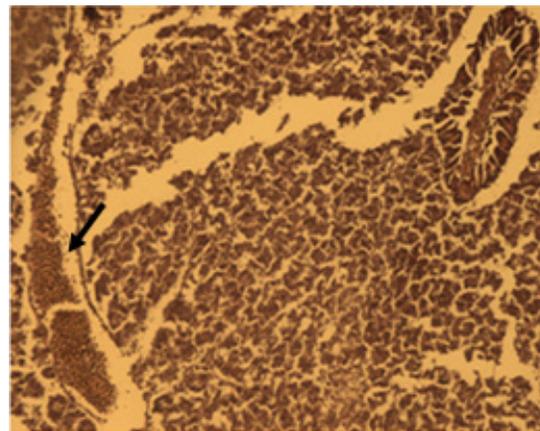


Figure 10. Section of liver showing vacuolar degeneration of hepatocytes, congestion of sinusoidal and hepatoportal blood vessels (H&E x 20).

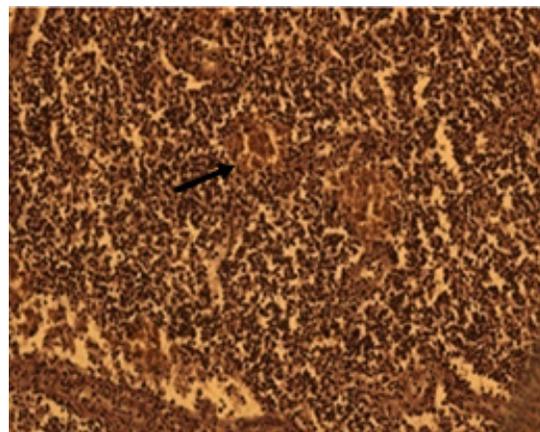


Figure 11. Section of Spleen showing Degraded RBC, disintegrated nucleus and necrotic tissue (H&E x 10).

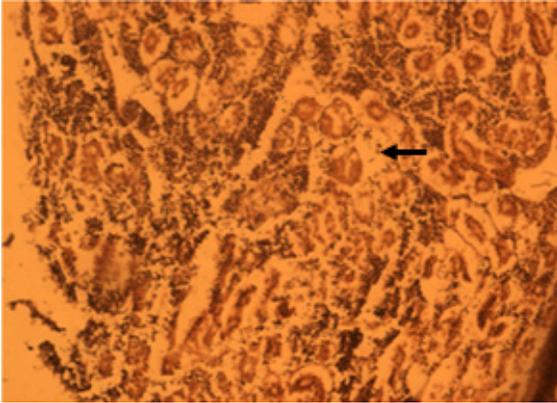


Figure 12. Section of kidney showing Necrosis and vacuolation of epithelial cells, lymphocyte proliferation with inflammatory changes (H&E x 10).

Alteration in testicular architecture with disruption, focal disintegration and necrosis of germinal epithelium was observed. Ovary showed drastic reduction in size with thin wall but no significant lesions were observed, atretic follicles less visible.

4. Discussion

Polyps are most common in the colon and may occur in esophagus, stomach and small intestine. Usually polyp begins as small elevations of the mucosa, enlarge by proliferation of cells adjacent to the mass and affects the traction on the luminal protrusion. In course of time it leads to inflammatory lesion with super imposed mucosal prolapse and include lamina propria fibromuscular hyperplasia, inflammatory infiltrates, erosion and epithelial hyperplasia with focal attenuated muscular mucosa. These adenomas are intra epithelial neoplasms become large lesions. The changes at the surface of the adenoma are presence of large nucleoli with eosinophilic cytoplasm and reduction in the number of goblet cells, containing prominent blood vessels derived from submucosa²². The above pathological manifestations were clearly observed in the present study. These are supported by similar observations such as gastric papilloma in *Epinephelus itajara*¹⁷, tumors of the gastrointestinal system in the rainbow trout¹⁸ and medaka *Oryzias latipes*¹⁹ gastric polyp in a wild-caught blue shark *Prionace glauca*²⁰.

The skin alterations correlate with a series of both dermis and epidermal alterations in fish integument exposed to waterborne toxicants²³. A reduction in skin

goblet cell size in fish, including flatfish, has already been found to occur as a result of different factors of bacterial infections²⁴ and exposure to sediment bound contaminants²⁵. As gill is the primary site of osmoregulation and respiration in fish which gets affected easily when exposed to infection, many aquatic pollutants¹ are assayed for their deleterious effects on the cellular ultra-structure of the gill epithelium^{26,27}. The present gill pathology such as hypertrophy and hyperplasia of lamellar epithelium leading to lamellar fusion would increase the thickness of free gas exchange, osmotic balance impairment, capillary hemorrhage, causing anoxic condition and alterations in carbohydrate metabolism affecting the general health of fish²⁸⁻³². The present muscle histology collaborate with findings of septicemia condition in systemic bacterial disease characterized by lesions in infected muscle^{33,34}.

The reduction in the size of fish liver revealed the ultra-structural changes characterized by severe enlargement and vacuolisation of hepatocytes as a signal of degenerative process that suggests metabolic damage³⁵. These changes are supported by similar studies on *Moronea mexicana*²¹, sea bass *Dicentrarchus labrax*³⁶, climbing perch *Anabas testudineus*³⁷, *Carassius auratus*³⁸, European eel *Anguilla anguilla*³⁹. Though the presence of lesions within the spleen where compatible with lesions associated with septicaemia, similar results were also reported in infected spleen showing symptoms of granuloma⁴⁰ and necrosis of sheathed arteries accompanied by haemorrhage, fibrin deposition, oedema in splenic pulp and sheathed tissue^{41,42}. Kidney lesions consisted lymphocyte proliferation and inflammatory changes, corroborating with reports on *Coregonus clupeaformis* peritubular and periglomerular cells, hypertrophic nuclei and haemolysis of erythrocytes induced by the various toxins⁴³. Such degenerative changes can lead to tissue necrosis with intense infiltration of mononuclear cells and intracytoplasmic granules⁴⁴⁻⁴⁶. Disruption of testicular and ovarian architecture is substantiated with reports on *Tilapia zillii*, *Glaucosoma hebraicum*, *Epinephelus septemfasciatus*, *Chalcalburnus mossulensis*, *Clarias gariepinus*⁴⁴⁻⁴⁹.

Microbiological studies revealed that *Klebsiella pneumoniae* infection in *N. japonicus* is the most common pathogen which develops polyps. *K. pneumoniae* is indeed the primary etiological agent of buccal mucosal polyps³, even metal pollutants may involve in the development of other pathological findings². Cytogenetic studies of *Nemipterus japonicus* with mucosal buccal polyps showed

polyploidy condition. Chromosomal composition revealed that $2n = 120$ to >200 chromosomes, were due to pathogenic effect⁴. Immunological assays revealed that metals may affect the lymphocyte directly by altering the synthesis of cellular DNA and thereby influence the immune responses⁵.

5. Conclusions

In the present study, we report an unusual polyp described as exophytic lesion which originate from surface or gastric pit mucous cells. There is a possibility that chronic inflammation may cause these neoplastic lesions in these fish. The reason for chronic inflammation is unknown at this time but there is a possibility of migration or encystations or chronic ingestion of irritating foreign material, an irritant chemical present in the habitats they live, or a combination of all the above factors. The results also reinforce suggestions that there is an evidence of an inherited susceptibility to adenoma with well recognized environmental risk factors, supports the hypothesis that genetic and environmental factors interact in the formation and transformation of polyps. The incidence of such neoplasms in wild fish populations are useful indicators of ecosystem contamination for a critical scientific analysis and exploration.

7. Acknowledgements

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