Research Article

Wallago attu (Bl.) and its Parasitic Monogenea *Mizelleus indicus* (Jain, 1957), Pandey *et al.*, 2003: A Model Towards Histopathological Studies for Host Parasite Interaction

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ABSTRACT

Host Parasite model study essentially reflect cruelty of parasite on the host through various means depending upon the site and degree of infection. Gill parasitic monogenea presents an excellent opportunity to look this in much more closer and specific way. *Wallago attu* (Bl.) was studied for histopathological damage caused by parasitic monogenea *Mizelleus indicus* (Jain, 1957), Pandey *et al.,* 2003. During the extensive study spread over three year, control and infected gills well examined through scanning electron microscopy (SEM) as well as light microscopic tools. The damage caused on the gills as observed on several occassions have been self explanatory for the decline in fish health under heavy infection. Observations made during the study were coroborated with known studies and discussed in detail.

Key words: Monogenea, *Mizelleus indicus,* Histopathology, Host-Parasite Model, Gill Parasite, Microscopy.

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INTRODUCTION

The gills in fishes are important organs of examination in disease diagnosis, because of their direct contact with the environment, which means they are sensitive to a number of irritants, parasites and pollutants present in the water.[1] The host and parasite system in case of monogeneans results into large-scale damage on the site of attachment. During ventilation in teleosts the water currents strike the edge of the primary lamella and pass between the secondary gill lamilla.[2] In order to prevent themselves from being swept away along with the water currents, monogeneans use their anchor and marginal hooklets as a mean of attachments to the host tissue. The attachment of monopisthocotylean monogeneans with small hamuli is enhanced by the tissue response of the host gills.[3] Presence of monogeneans has been reportedly found to be associated with the hyperplastic response, lamellar fusion, haptoral embedding in tissue, gill erosion of various degrees.[4-17] High densities of monogeneans on gills cause severe necrosis of the gill tissues, possibly resulting in suffocation.[18] They also postulated that the 'sleepy grouper disease' is a syndrome involving many pathogens including monogeneans. The devastating impact of monogeneans can loss of more than 50 tonnes of fishes in Australia alone due to a single monogenean species.[19] Hyperplasia of the gill epithelium and structural disorganization of secondary lamellae was seen diffusely in the gills, leading to fused lamellae in the gills.[14] The histopathological alterations due to *Diplodocus paradox* lead to hyperplasia followed by complete sloughing of secondary gill lamellae.[15] *Sparus aurata* with history of loss of appetite and sluggish movement with increased breathing frequency, in terms of monogenean revealed different pathological changes.[16] Necrosis of the gill lamellar epithelium,

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hyperplasia of mucous secreting cells and focal areas of denuded epithelium were the most common.[16]

As far as histopathological changes as such in piscine gills is concerned it has not been studied extensively. Thus, with a view to study the histopathological changes in gill filaments of *W. attu* (Bl. and Schn.) due to infection of *M. indicus,*[20,21] the present investigation was started.

MATERIALS AND METHODS

a. Collection and Identification of the Piscine Hosts - The fish *Wallago attu* (Bl. and Schn.), for the present study were obtained from the suppliers in local fish markets of Meerut, Uttar Pradesh, India. On the basis of experiment due care was taken to ensure the uniformity of the source of host habitat, identification of host was carried out with the help of classical work.[22,23] The fishes were immediately examined at Laboratory, Department of Zoology, Ch. Charan Singh University Meerut, Uttar Pradesh, India.

b. Collection of the Parasitic Monogeneans - For the collection of parasites protocol standarised in the lab *viz.,* freezing technique, chloretone technique.[24-32]

Light Microscopy: At the outset, parasites were fixed.[26,33,34] Chitinoid hard parts of the parasites was made in temporary glycerine mounts.[34,35] The measurements were recorded in millimetres with the help of calibrated ocular graticule and Camera Lucida projections and were expressed as minima to maxima ranges. The drawing of the holotype was prepared using Camera Lucida. The identification of the monogeneans has been made with the help of classical work.[21,36]

Electron Microscopy: Besides this, fine morphological studeis were as per method.[4,5] For the study of detailed pathomorphological changes through SEM, gills were processed as per guidelines given by Electron Microscope facility, AIIMS, New Delhi, the SEM viewing/ photography was carried out on LEO 435 VP Scanning Electron Microscope at Electron Microscope Facility, AIIMS, New Delhi.

Histopathology: For light microscopic, pathomorphological studies, entire gill filaments, gill arches of infected and non-infected gills were stained in Acetoalum Carmine, dehydrated through ascending grades of alcohol, cleared in Xylene and mounted in Canada Balsam/DPX. Histopathological, studies of gills of infected and uninfected fish specimens were sectioned in Paraffin Wax and stained in Hematoxylin/Eosin, dehydrated, cleared in Xylene and mounted in Canada Balsam/DPX. Later the slides were observed under the Microscope and photographed.

OBSERVATION

The site of attachment of *M. indicus,*[20,21] is the gill filaments as observed during the entire study (Figure A (1-10)) and also reported by other workers. Flap like folds of secondary gill lamella occur on either side of primary gill lamellae. Microvilli are sparse or absent on the secondary gill lamellae (Figure A (1-2)). *M. indicus,*[20,21] are located between two successive secondary gill lamellae, where they are securely anchored to their site of attachement with the help of haptoral sclerites. *M. indicus*[20,21] uses its robust dorsal anchors (Figure A (3-4)) for attachement by boring them deep into the gill filaments. The ventral anchor being relatively smaller in size causes smaller bores. Whereas, the fourteen marginal hooklets, aid *M. indicus*[20,21] in attachment by strucking here and there in the nearby gill tissues. At number of occasions, the haptoral anchors and marginal hooklets were found to be embedded very deep into the gill tissues.

The surface of the haptor is closely apposed to the surface epithelium of the gill filaments (Figure A (5-7)). Whereas, the anchors are embedded in the gill tissue. Rupture of membrane together with erosion and deformation occur on the gill filament in correspondence with the points of attachment of anchors and marginal hooklets, which leaks host blood cells. *M. indicus,*[20,21] infection was observed to result into increased mucus secretions and destruction of gill structures (Figure A (5-9)). The hamuli can perforate the gill tissue and reach the gill cartilage. Surface deformation and apparent swelling of the epithelium of the secondary gill lamellae were observed at the site of attachment. Severe haemorrhages and extensive hyperplasia of the gill epithelium were seen and in many cases at the point where haptor was embedded in the gill tissue.

SEM studies of gills together with light microscopic study of sections revealed that *M. indicus,*[20,21] was often surrounded and partly embedded by extensive hyperplasia of gill filaments (Figure A (8-9)). The parasites were widely distributed over the gill filaments, but aggregations of parasites were often seen on the filaments towards the bend of the gill arch.

Infection with *M. indicus,*[20,21] on the gills of *W. attu* (Bl. and Schn.) elicit a host reaction presenting a deformity of the gills associated with bruises, inflammation and leakage of host blood cells at the site of attachment on the gill epithelium (Figure A (10-12)). Such kind of inflammatory responses in a way may become advantageous to parasite in securing attachment by haptor. Comparison between control and infected gill filaments revealed that the gills with heavy infection have the

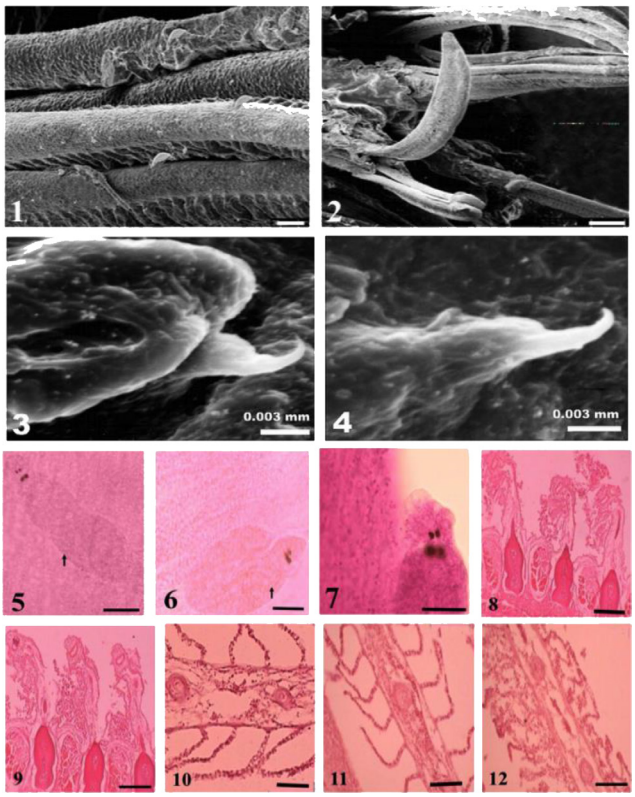

Figure A: Scanning Electron Micrograph (SEM) and Light Microscopy (LM) images of gills, parasite and cross sections of gills for examination of histopathology and pathomorphology (1-2. SEM of gills showing worms and pathomorphology; 3-4. SEM of worms focusing on hooks responsible for gill puncture; 5-7. LM of gills showing attached worms and pathomorphology; 8-9. LM of gills showing cross section of gills; 10-12. LM of gills showing cross section of highly infected hosts).

condition of erosion of secondary gill lamellae, lamellar hyperplasia and lesions caused by the lamellar fusion. At several occasions gill filaments were found to be completely eroded from the gill, especially in case of those fish which were found dead or very highly infected. Moreover, at some places mild degree of epitheliocytosis (Figure A (2,6,12)) is apparently visible which is characterized by enlargement of epithelial tissue at the site of injury resulting into tumorous growth.

DISCUSSION

Hypoplastic changes in infected gills, erosion of secondary lamellae and presence of lesions has been the most prominent histopathological changes caused by infection of *M. indicus,*[20,21] which corroborates with earlier findings.[1,11,37] As far as cause for the development of hyperplasiasis is concerned hyperplastic lamellae are those from which gill flukes have fed by breaching the epithelial tissue and blood vessels.[37] Signals of hyperplastic changes comes from possibly secretory and excretory products of parasites.[1] Which is aggreable because monopisthocotleans during feeding and attachment use to discharge histolysins which might prove to be hyperplastic signals. At several occasions gill filaments were found to be completely eroded from the gill especially in those fishes which were found dead or very highly infected. Erosion of gill filaments resulted in the extensive loss of respiratory surface area. Examination of samples of host from the site of natural mortality associated with monogenean infection revealed the complete erosion of gill lamella causing extensive loss of respiratory surface area, ultimately high rate of mortality. High densities of gill monogeneans cause severe necrosis of the gill tissues possibly resulting in suffocation.[18] A mild to moderate epitheliocystis was detected in the gills of amberjack affected by gill flukes.[11] Epitheliocystis not found in amberjack affected by mass mortalities most probably caused by *Zeuxapta seriolae*.[9] Epitheliocystis has been reported in a number of fish species from around the world.[38] In Australia this condition has been reported from various fish species including sea-farmed Atlantic salmon in Tasmania[39] and wild marine fish in New South Wales.[40] Epitheliocystis is usually regarded as a benign condition and has no clinical significance in Atlantic salmon cultured in Tasmania.[39] Epitheliocystis was also observed in Jack Mackerel and a long-finned Pike.[41] During the study epitheliocytosis has also been seen in gills of some infected fishes. But exactly at this moment it can not be said that this epitheliocytosis is a outcome of monogenean infection as there are several other agents like virus as also been reported.[42,43]

Intraepithelial cysts of on *Piaractus mesopotamicus* and *Prochilodus lineatus* by monogenean and mixosporean cysts caused lamella dilation and deformity of adjacent lamellae, hyperplasia of the gill epithelium and structural disorganization of secondary lamellae was seen diffusely in the gills, leading to fused lamellae in the gills, in few cases, mononuclear inflammatory cells and hemorrhagic focal points distally in the lamellae.[14] The histopathological alterations due to *Diplodocus paradox* lead to hyperplasia followed by complete sloughing of secondary gill lamellae, pathological alterations such as proliferative, necrotic as well as degenerative changes in the epithelium of gill filaments, aneurism in secondary lamella, cartilaginous tissues of gill filaments displayed severe proliferation causing deformity and thickening of gill filaments.[15] *Sparus aurata* with history of loss of appetite and sluggish movement with increased breathing frequency, in terms of monogenean *Furnestinia echeneis, Encotyllabe spari, Sparicotyle chrysophrii* and *Choriocotyle chrysophrii,* revealed different pathological changes in the affected branchial tissue depended on

the type of the detected monogenea.[16] Necrosis of the gill lamellar epithelium, hyperplasia of mucous secreting cells and focal areas of denuded epithelium were the most common lesions noticed at the sites of parasites attachment.[16] It is proposed that due to primary infection of monogeneans, injury is inflicted through which entry of virus took place that might have developed into epitheliocytosis. The damage caused by the worms under heavy infection is directly or indirectly contributing to onset of secondary infection and related histopathological modifications. The present model of fish and its parasitic monogenea may be treated as a reference model for histopathological studies on host parasite interaction.

CONCLUSION

Therefore, the host and parasite relationship can determine the fate of their life *vis-à-vis* degree of infection, immunity, pathogenecity, seasonal diversity, environmental factors may be few among them. A simple model in the from of external gill parasite affecting freshwater fish may be well considered for variety of evaluations, understanding of biological phenomenon, interrelationships and above all disease management approach to be the most important one. Scanning Electron Microscopy, Light Microscopy can be specifically helpful in investigation and establishment of facts through scientific treatment of concept.

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CONFLICT OF INTEREST

The authors declare no Conflict of interest.

ABBREVIATIONS

SEM: Scanning Electron Microscopy; **LM:** Light Microcopy; **DPX:** Dibutylphthalate Polystyrene Xylene; **CSIR:** Council of Scientific and Industrial Research; **JRF:** Junior Research Fellow; **SRF:** Senior Research Fellow; **AIIMS:** All India Institute of Medical Sciences.

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