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Myxosporidian infection of gills and skin among carp from nursery ponds in Bangladesh: histopathology

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ABSTRACT

Histopathological studies of gills and skin of juvenile carp infected with myxosporidian protozoans in two fish farms (Trisal Government fish farm and Jhalak Private fish farm) of Mymensingh district in Bangladesh were carried out during the period September to December 1994. A total of 140 carp ranging from 4 to12 cm in total length were examined and comprised 70 *Labeo rohita* (*L. rohita*), and 70 *Cirrhina mrigala* (*C. mrigala*). Samplings were carried out at 15-day intervals. Pathological symptoms were more prominent in carp at the Government fish farm (GFF) than in those at the Private fish farm (PFF). Marked pathological changes were limited to the gills and skin of *C. mrigala* at the GFF in December 1994. Hyperplasia and hypertrophy, with presence of numerous inflammatory cells and an accumulation of blood cells, were observed at the base of secondary gill lamellae of *C. mrigala* at the GFF. Myxosporidian cysts, identified as *Myxobolus* spp. and full of mature spores, were attached to the secondary gill lamellae. A significantly increased number of cysts were recognized

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in the gills of *C. mrigala* at the GFF compared to those of *L. rohita* at the PFF. The skin of *C. mrigala* at the GFF exhibited an increased number of pathological changes. Epidermis and dermis sloughed off, the dermal layer split into a few parts, and Myxosporidian cysts full of mature spores appeared in the skin of *C. mrigala*. At the PFF, the skin of *L. rohita* was less infected than *C. mrigala*. Apparently healthy fish fry was found to be free from cysts. No significant differences of water quality parameters were recorded at either fish farm. Carp at the PFF were less infected than those at the GFF, probably due to its better management practices.

Key words: histopathology, carp, myxosporidia, Bangladesh

Introduction

The healthy population of any species depends on control of diseases and the maintenance of a healthy relationship between living creatures and their environment. Health care is therefore based on a knowledge of organisms, their ecology and the application of this knowledge in the control of diseases (SNIESZKO, 1983).

Parasites and diseases are the most serious limiting factors in aquaculture in Bangladesh because fish are usually cultured in high density in a restricted water body, where fish pathogens can easily be transmitted between fish. Myxosporidian protozoan is one of the most common groups of parasites infecting juvenile carp in Bangladesh, causing serious damage to and mortality among young carp. The importance of myxosporidian parasitism of fish stocks has come to light in recent years. Reports on pathological symptoms, the mortalities caused by them, as well as their biology, has been reviewed by MITCHELL (1977), and SEENAPPA and MANOHAR (1981).

Nursery ponds for rearing fry and fingerlings are comparatively limited in Bangladesh. Management practices in the available nursery ponds of both the Government and private sectors are not yet at a standard level. Fish farmers often raise questions about the quality of fry obtained from nursery ponds, where they have observed reduced growth performance. The poor growth of juvenile carp depends on many factors. Among others, the presence of ecto- and endoparasites is one of the major problems. Protozoan and monogenean ectoparasites are common in juvenile carp in nursery ponds. High mortality rates caused by myxosporidians infection in the gills have raised serious concern among fish farmers.

However, very little is known about the histopathology of gills and skin of fish in the Indian subcontinent. A histological study on the major carp, especially on *Catla catla*, has been reported previously (SANAULLAH and AHMED, 1980). There are alarming economical losses due to myxobolus spp. infestation of major carp in the nursery ponds of Bangladesh. This was also reported by SANAULLAH and AHMED (1980).

DAVIS (1923) studied the sporulation and development of cysts of myxosporidian species. Histological changes in the gills of tench, *Tinca tinca*, caused by *Myxobolus ellipsoides* has previously been reported (AISA, 1972). Pathoanatomy and histopathology of some vital organs of the catla affected by *Myxobolus* spp. were studied by DEY et al. (1988).

BALL et al. (1990) reported a light and scanning electron microscopic study of the gills of diseased pure crucian carp (*Carassius carassius*) from the Thames Water Authority Area, U.K., and revealed the existence of a protozoan parasite in the gills.

Histopathological knowledge has been successfully used to diagnose diseases of aquatic organisms throughout the world. In Bangladesh, such techniques have yet to be applied in the diagnosis of diseases in an aquaculture system. The present work was therefore undertaken to investigate the histopathological changes of gills and skin of juvenile carp *Cirrhina mrigala* and *Labeo rohita* to clarify the nature of damage caused by myxosporidian parasites.

Materials and methods

This research was conducted during the period September to December 1994 at the optimum time of stocking of fingerlings in nurseries. Two carp species known as rohu (*Labeo rohita*) and mrigal (*Cirrhina mrigala*) were sampled from two different fish farms (Trishal government fish farm and Jhalak private fish farm) at fortnightly intervals (Fig. 1). A total of 140 fish ranging from 4 to 12 cm in total length were selected for this study. Living fish were collected by seine net. Water quality parameters such as temperature, dissolved oxygen content (DO) and pH were recorded during each visit.

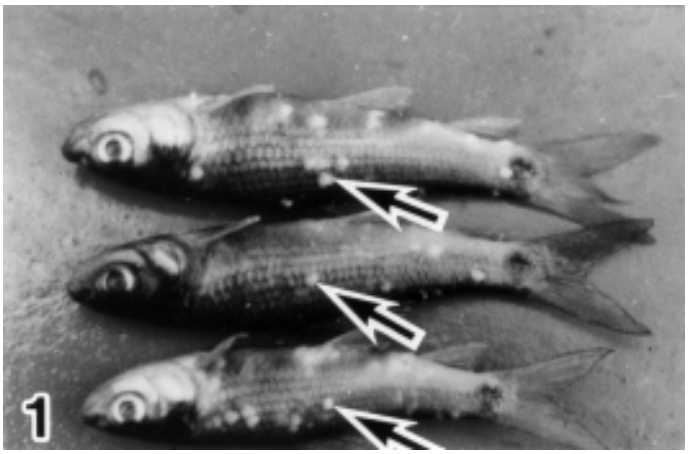

Fig. 1. Photograph showing Myxosporidian cysts (arrows) on body surface of juvenile carp (*Labeo rohita*) in Bangladesh

All the fish were killed by a blow to the head. Ten fish of each species were examined to study the presence of any external parasites, or any other abnormalities, during the sampling period. For histological study, gills and skin of about 1 cm^3 each in volume were excised and immediately preserved in 10% buffered neutral formalin. The amount of fixatives was 10 times volume to the bulk of the tissue fixed (HUMASON, 1979). The samples were then taken in a perforated plastic holder covered with perforated steel plates. Dehydration, clearing and infiltration processes were carried out in an automatic tissue processor using a series of graded ethanol, two changes of chloroform and, finally, in three consecutive series of molten wax. The samples were then embedded in paraffin wax and sectioned at 5 μm thickness. Sections were stained with Meyer's hematoxylin and eosin (GRIDLEY, 1960), and mounted with Canada balsam. The prepared sections were then examined under a light microscope (Olympus, Japan) for general histological studies. For scanning electron microscopy the samples were dehydrated in a series of graded ethanol and acetone, dried by critical point drying through liquid CO_2, mounted on a metal stub, coated with a thin conductive film of gold and examined using a JSM-T 300 Scanning Electron Microscope (SEM) at 15 kV.

Results

Water quality parameters, such as temperature, dissolved oxygen, (DO) and pH, as noted during the sampling period, were almost the same and were found to be in a suitable range for fish culture at both farm ponds (Table 1).

Table 1. Water quality parameters at farm ponds

Sampling time	Parameters					
	Temp. (°C)		pH		Dissolved oxygen	
	Private farm	Government farm	Private farm	Government farm	Private farm	Government farm
September`94	32.0-33.4	32.0-35.0	7.4-8.0	7.0-7.2	4.2-5.0	4.2-4.5
October`94	33.0-35.0	30.0-30.5	7.0-7.2	7.5-8.0	3.0-3.5	3.5-5.0
November`94	25.0-25.5	25.5-28.0	7.0-7.4	7.4-8.0	4.1-4.2	3.0-4.5
December`94	25.7-24.5	22.7-25.0	7.0-7.2	7.5-8.2	3.5-5.0	4.5-5.0

Histopathological changes in gills of and skin of rohu (*L. rohita*) and mrigal (*C. mrigala*) juveniles in both farms are shown in Table 2.

Gill pathology of *L. rohita*

Government farm. In September 1994, less pathological changes were observed in the gills. Both primary and secondary gill lamellae were arranged systematically. A very few myxosporidian (*Myxobolus* spp.) cysts were attached to the secondary gill lamellae. However, in December 1994 large myxosporidian cysts with mature spores were attached to the primary gill lamellae and occupied at least 5-6 secondary gill lamellae. Numerous blood cells accompanied by inflammatory cells appeared at the base of secondary gill lamellae (Fig. 2a).

Private farm. No infestations were noted during the period from September to October 1994. Primary and secondary gill lamellae were observed to be normal.

Skin pathology of *L. rohita*

Government farm. No remarkable pathological changes were found during the period from September to October 1994. However, towards the months of November and December, there was a tendency for the dermis to split into a few parts, the dermal cell layer sloughed off and melanocytes were seen at the base of the dermis.

Private farm. Epidermal and dermal parts sloughed off in most regions. The dermal layer of the skin split into a few parts in December 1994.

Table 2. Pathological changes in the various organs of *L. rohita* and *C. mrigala* during the experimental period (Sept-Dec 1994)

Month	Species	Government fish farm	
		Gill	Skin
September	*Labeo rohita*	Gill lamellae were arranged systematically. Myxoporidian cysts were attached to secondary gill lamellae.	Epidermis was lost and a part of dermis sloughed off.
	Cirrhina mrigala	Numerous myxospridian cysts with inflammatory cells were observed in both primary and secondary gill lamellae. Blood cells also attached to the base of secondary gill lamellae.	Epidermis was separated from dermis. Part of dermis sloughed off in many regions.
October	*Labeo rohita*	Gill lamellae were more or less normal. A large myxosporidian cyst occupied almost whole length of secondary gill lamellae.	Part of epidermis was separated from the dermis.
	Cirrhina mrigala	Secondary gill lamellae were lost in some regions. A cyst appeared at the base of secondary gill lamellae. Numerous blood cells were also observed in secondary gill lamellae.	Large myxosporidian cysts at the external part of epidermis, with fully mature spores were observed. Cyst wall was ruptured.
November	*Labeo rohita*	Large myxoporidian cyst with mature spores was observed. The cyst occupied the 5-6 secondary gill lamellae. Numerous blood cells appeared at the base of secondary gill lamellae. Gill lamellae were lost in some regions.	Epidermis was lost, dermis split into a few parts, and dermal cells sloughed off. Melanocytes were present in the dermal region.
	Cirrhina mrigala	Gills were affected by cysts. Gill lamellae were irregularly arranged. Numerous inflammatory cells, together with blood cells, were found along the secondary gill lamellae.	Part of the epidermis was lost. A cyst was found in the epidermis.
December	*Labeo rohita*	Both primary and secondary gill lamellae were systematically arranged. A large myxosporidian cyst was found along the 5 to 6 secondary gill lamellae. Numerous blood cells together with inflammatory cells were present at the base of secondary gill lamellae.	Dermis split into a few parts. Dermal cells sloughed off.
	Cirrhina mrigala	Marked pathological changes were observed. Numerous cysts were present in primary and secondary gill lamellae. Hyperplasia and hypertrophy in both the gill lamellae were evidenced. Numerous inflammatory cells and blood cells were found.	Cysts attached to dermal folds of the skin. The cysts were filled with mature spores. Epidermal and dermal parts of the skin were separated.

Gill pathology of *C. mrigala*

Government farm. Gills of *C. mrigala* were severely infected in December 1994 and maximum infestations were recorded at that time. Myxosporidian cysts (Myxobolus spp.) with inflammatory cells were found attached to both primary and secondary gill lamellae. Numerous blood cells also appeared at the base of secondary gill lamellae. Gill lamellae were hypertrophied and hyperplasid. A part of secondary gill lamellae was found to be absent in some regions (Fig. 2b).

Table 2. (continued) Pathological changes in the various organs of *L. rohita* and *C. mrigala* during the experimental period (Sept-Dec 1994)

Month	Species	Private fish farm	
		Gill	Skin
September	*Labeo rohita*	Primary and secondary gill lamellae were normal. No infestations were noted.	Epidermal and dermal parts of skin were absent in most regions. Dermis split into a few parts.
	Cirrhina mrigala	Part of secondary gill lamellae was lost. Large myxosporidian cytes were observed in secondary gill lamellae.	Epidermises were lost in many regions. A few parts of dermis slightly sloughed off. Many inflammatory cells were observed in both epidermal and dermal layers.
October	*Labeo rohita*	Primary gill lamellae were hypertrophied and hyperplasid. An oval-shaped cyst occupied the whole length of secondary gill lamellae.	Epidermis was lost and split into a few parts in some regions.
	Cirrhina mrigala	Primary and secondary gill lamellae were lost in many regions. Several myxosporidian cysts were attached side by side with secondary gill lamellae. An accumulation of blood cells was also observed.	Epidermis and dermis were separated from each other.
November	*Labeo rohita*	Primary gill lamellae were slightly hypertrophied. Gill lamellae were more or less systematically arranged.	Epidermis and dermis were systematically arranged.
	Cirrhina mrigala	Both primary and secondary gill lamellae were severely affected by cysts. Gills were clubbed to each other in many regions.	Epidermal and dermal parts of the skin were lost in many regions.
December	*Labeo rohita*	Less infection was observed in gill lamellae and was systematically arranged.	Epidermis and dermis were lost in most regions. Dermis was split into a few parts.
	Cirrhina mrigala	Part of secondary gill lamellae was lost in many regions. A myxosporidian cyst was found in the secondary gill lamellae.	Marked pathological symptoms were observed in epidermal and dermal layers of the skin. Part of epidermis was lost. Dermal layer sloughed off.

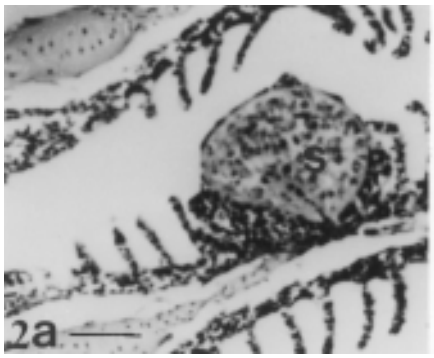

Fig. 2a. Photograph showing gills of *L. rohita*. A protozoan cyst full of mature spores is present at the base of the secondary gill lamellae. H&E; ×135; scale bar = 50 μm.

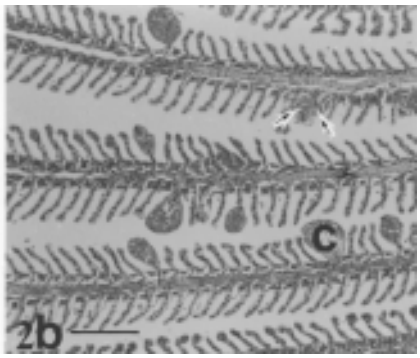

Fig. 2b. Photograph of *C. mrigala*. Numerous myxosporidian cysts (c) with inflammatory cells (small arrows) are present in both primary and secondary gill lamellae. H&E; ×135; scale bar = 50 μm.

Private farm. Gills of *C. mrigala* at PFF were comparatively less infected with those at the GFF. Arrangements of gill lamellae were normal during the period September to October 1994. Several secondary gill lamellae with blood cells were observed in some regions. Both gill lamellae were increasingly affected towards the months of November/December 1994. Gills were found clubbing to each other in some instances.

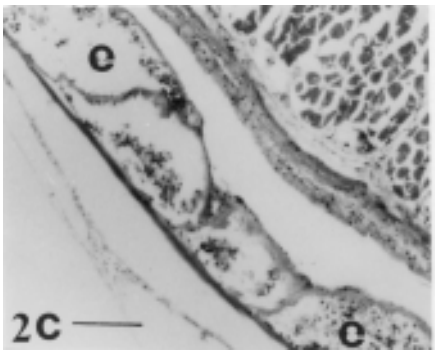

Fig.2c. Photograph showing skin of *C. mrigala*. Many irregularly shaped cysts (c) are seen along the side of dermal folds. H&E; ×135; scale bar = 50 μm.

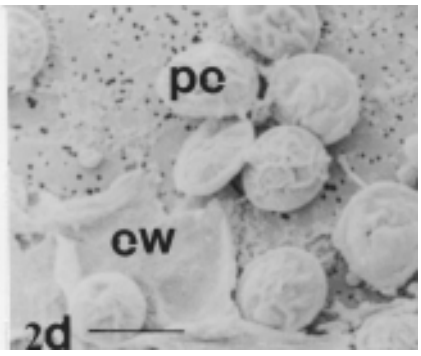

Fig.2d. Scanning electron micrograph of a Myxobolus spp. A polar capsule (pc) with ruptured cyst wall (cw) is seen. ×18,000 scale bar = 10 μm.

Skin pathology of *C. mrigala*

Government farm. In October 1994 a large number of cysts were found in the dermal fold of the skin (Fig. 2c). The cysts, consisting of fully mature spores, were close to rupturing their walls. In December 1994 many irregular cysts attached to the dermal fold of the skin, side by side. All cysts contained fully mature spores (Fig. 2d). The epidermises of the skin sloughed off and separated from the dermis in some instances. Melanocytes were present in the dermal layer of the skin.

Private farm. In September 1994 epidermises were lost in many parts of the skin. In some regions the dermal part of the skin slightly sloughed off. Numerous inflammatory cells were present in both the epidermal and dermal layers of the skin. Several pathological changes were also observed in December 1994. Among these, sloughed-off materials from the epidermis and dermis of the skin were recorded.

Scanning electron microscopy. By scanning electron microscopy, *Myxobolus* spp. with a distinct polar capsule, was clearly visible. The *Myxobolus* spp. was observed to be released from the ruptured cyst wall (Fig. 2d).

Discusssion

Maintenance of water quality parameters within the normal range is one of the most important factors for culturing fish. During the investigation the water quality parameters were almost similar at both the GFF and the PFF. However, the water level in both ponds receded during the winter season. Oxygen content and water temperature fluctuated from time to time.

CHANDRA (1987) reported that unfavourable environmental conditions could cause a variety of diseases in fish. It has also been reported that fingerlings are more susceptible to disease (CHANDRA, 1987). The occurrence of myxoboliasis in the present study might be due to unfavourable environmental conditions such as reduced water level, and comparatively low oxygen content associated with high water temperature. The probable cause of higher infestations by *Myxobolus* spp. in mrigal could be the existence of oligochaetes, as they need oligochaetes as an intermediate

host to complete their life cycle. Oligochaetes live in the bottom mud of ponds. Therefore, the bottom feeding mrigal easily be infected by transmission from the oligochaete intermediate hosts. WOLF and MARKIW (1984) claimed that *Myxosoma cerebralis* required an oligochaete intermediate host for completion of their life cycle.

Gills were found to be more infested by *Myxobolus* spp. Such higher infection in the gills might be due to the suitable habitat of myxobolus spp. The gills of fish are an important site for disease production, because they are a rich source of blood, an important media for the infectious agents. Since gaseous exchange takes place through the gills, they may easily become contaminated from external sources. LAGLAR et al. (1963) reported that gaseous exchange and other solutes between blood and water take place in the gill lamellae. Maximum infection was found in the gills, but not in the skin. Histopathological changes derived from *Myxobolus* spp. infection in the gills of *L. rohita* and *C. mrigala* are in accord with the findings of DEY et al. (1988), SANAULLAH and AHMED (1980), and DYKOVA and LOM (1978). *Myxobolus lintoni* caused marked changes in the epidermis and hypodermis of the skin of the host *Cyprinodon variegates*, characterized by invasion of fibroblasts (DYKOVA and LOM, 1978). In the present study, the cysts of *Myxobolus* spp. were found at the tip of the primary and secondary gill lamellae. SANAULLAH and AHMED (1980) reported that parasitic cysts were observed in the distal tip of the primary gill lamellae along their length. The cysts in the proximal region of the filaments were generally much smaller than those in the distal region. The cysts in the present study were round in shape and located along the margin of skin folds. This finding is completely in accordance with the report by HALDER et al. (1983).

Although most of the young carp of the nursery ponds were apparently healthy, a greater percentage of fish were affected by gill parasites, especially myxosporidians spp. They were identified by histological studies. It has been assumed that there is every possibility for them to be involved with infestation in other major organs such as kidney, liver, spleen, intestines, and so on. The infestations in these organs were not considered in the present experiment. Therefore, further research is needed to provide adequate evidence of the cause of diseases and their treatment.

Conclusions

Fish at the PFF in the present study were less infected because of better management practices in comparison with those at the GFF. More precautionary measures should be taken for *C. mrigala* and management practices at the GFF should be improved. In a culture system, more attention should be paid to the situation after rain falls in the rainy season, when the chances of infection have increased for farmed fish.

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References

AISA, E. (1972): Observazioni su una missoboliosi delle Tinche. Parasitol. 14, 219-225.

BALL, S. J., D. A. ZACHAREK, A. E. DEURSLEY, J. F. MOORE (1990): Gill disease of crucian carp *Carassius carassius* associated with an unidentified protozoan. Dis. Aquat. Org. 8, 151-153.

CHANDRA, K. J. (1987): Fish health monitoring and control of disease. In training manual of training on integrated farming to the upazila fisheries officer. DOF, Bang. 1, p.155.

DAVIS, H. S. (1923): Studies on sporulation and development of the cysts in a new species of *Myxosporidia*, *Lentospora ovalis*. J. Morphol. 37, 425-456.

DEY, R. K., D. KUMAR, B. K. MISHRA (1988): Tissue level reactions in the Indian major carp *Catla catla* due to *Myxobolus* spp. infection. Asian. Fish. Science 1, 117-122.

DYKOVA, I., J. LOM (1978): Histopathological changes in fish gills infected with *Myxosporidian* parasites of the genus *Henneguya*. J. Fish. Biol. 12, 197-202.

GRIDLEY, M. F. (1960): Manual of histologic and special staining techniques. McGraw-Hill Book Co. Inc. New York.

HALDER, D. P., M. K. DAS, B. K. SHARMA (1983): Studies on protozoan parasites from fishes, four new species of the genera *Henneguya thelohan, Thelohanellus kudo, Myxobolus butschli.* Arch. parasitol. 127, 283-296.

HUMASON, G. L. (1979): Animal Tissue Techniques. W. H. Freeman and Co. San Francisco.

LAGLAR, K. F., J. E. BARDACH, R. R. MILLER (1963): Ichthyology. John Wiley and Sons Inc. London.

MITCHELL, L. G. (1977): Parasitic protozoa. Academic Press. New York. 2, p. 115.

SANAULLAH, M., A. T. A. AHMED (1980): Gill myxoboliasis of major carps in Bangladesh. J. Fish. Dis. 3, 349-354.

SEENAPPA, D., L. MANOHAR (1980): Two new species of Myxobolus - (Myxosporidea: Protozoa) parasitic on *Cirrhina mrigala*-(Hamilton) and *Puntius curmuca* - (Hamilton). Curr.-Sci. 49, 204-206.

SNIESZKO, S. F. (1983): Diseases of fishes. Research and control. Fisheries 8, 20-22.

WOLF, K., M. E. MARKIW (1984): Biology contraveness taxonomy in the *Myxozoa*. A new discoveries show alteration of invertebrate hosts. Science 225, 1449-1452.

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SAŽETAK

Patohistološka istraživanja škrga i kože mladih šarana invadiranih mikrosporidijima uzgajanih u dva ribnjaka na području Mymensingha u Bangladešu provedena su u razdoblju od rujna 1994. do prosinca 1994. Ukupno je bilo pretraženo 140 šarana (70 vrste *Labeo rohita* i 70 *vrste Cirrhina mrigala*) veličine od 4 do 12 cm. Uzorci su uzeti svakih 15 dana. Patološki su znakovi bili učestaliji u šarana podrijetlom iz državnih ribnjaka u odnosu na one iz privatnih ribnjaka. Najveće su promjene utvrđene u vrste *C. mrigala*. Hiperplazija i hipertrofija te veliki broj upalnih stanica utvrđen je na bazi sekundarne škržne lamele u iste vrste šarana. Ciste s potpuno zrelim sporama identificirane su kao *Myxobolus* sp. Iste su ciste češće pronađene na škrgama šarana podrijetlom iz državnih ribnjaka. U šarana podrijetlom iz državnih farmi učestalije su utvrđene promjene i na koži, i to u epidermisu i dermisu. Ciste nisu utvrđene u mlađi. Tijekom istraživanja nisu utvrđene nikakve razlike u kakvoći vode. Na osnovi nalaza može se smatrati da je manja invadiranost šarana podrijetlom iz privatnih ribnjaka uvjetovana boljom tehnologijom.

Ključne riječi: patohistološki nalaz, šaran, miksosporidiji, Bangladeš