PREVALENCE AND HISTOLOGICAL CHANGES OF MYXOSPOREAN PARASITES IN THE KIDNEYS OF *CIRRHINUS MRIGALA*, *LABEO ROHITA* AND *LABEO CATLA* (HALMILTON, 1822) FROM ALONE FISH CULTURE POND, MONYWA TOWNSHIP

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Abstract

Myxosporean parasites were important disease in culture fish species due to decrease fish production. Myxosporean infection in the kidney of three carp species, *Labeo rohita*, *Cirrhinus mrigala* and *Gibelion catla* was conducted from Alone fish culture pond, Monywa Township from June 2019 to May 2020. Amounted to 14 species of myxosporean parasites were observed. The total 90 fish specimens of three carp species, *Cirrhinus mrigala*, *Labeo rohita*, and *Gibelion catla* were studied for the prevalence of myxosporean parasites and histological changes in the kidneys of these species. Seven species of *Myxobolus*, and one species of *Thelohanellus* in *Cirrhinus mrigala*, three species of *Myxobolus* and one species of *Thelohanellus* in *Labeo rohita* and two species of *Myxobolus* in *Gibelion catla* were observed. The prevalence of myxosporean parasites of three carp species, *Cirrhinus mrigala*, *Labeo rohita* and *Gibelion catla* were found 83.33 %, 53.33 % and 13.33 % respectively. Histological changes were studied in infected kidneys of three carps, the large amount of matured myxosporean parasites were observed in the epithelial cells of renal tubules, in the blood corpuscle, and glomerulus of kidney. Among these three carp species, *Cirrhinus mrigala* was infected by more diverse species of myxosporean parasites and appeared more susceptible than the other two carps.

Key words: Three carp species, kidneys, myxosporean parasites, prevalence, histological changes

Introduction

Myxosporean parasites are abundant and diverse group of parasites and they cause diseases in a large variety of economically important fishes in both the wild and aquaculture fisheries industries. They have been also found in platyhelminthes, reptiles, amphibians, mammals and were also detected in fecal sample of human beings (Boreham *et al.*, 1998). They are multicellular organisms and consisting of one to twelve polar capsules. Several myxosporean infections of cultured fish were reported to be pathogenic. Most notorious is the whirling disease of trout, manifected by skeletal deformites, which is also claimed to have been introduced with rainbow trout into South Africa (Van Wyth, 1968). In farmed carp, *Myxobolus* spp. caused locomotory disturbances coupled with emaciation, and sunken eyes in brain infections (Dykova *et al.*, 1986). Members of phylum Myxozoa cause some of the most common and important parasitic diseases of fishes. Several species are known to cause serious losses in pisiculture (Lom and Dykova, 2006).

The protozoan parasite *Myxobolus* is often seen in the fingerlings causing morbidity and mortility in the carp polyculture system (Mukherjee *et al*., 2000). Parasites and diseases are one of the limiting factors in aquaculture, especially in the farms where fish are usually cultured in high density in restricted water body because fish pathogens can easily be transmitted among fish (Moe Kyi Han, 2006).

Myxozoans that play a role in cause diseases of commercially important fish. *Myxobolus* species is usually causes cutaneous myxosporidiasis in common fishes (Szczepanik *et al.*, 2010). In farm carp, *Myxobolus* spp. caused locomotory disturbances coupled with emaciation and sunken eyes in the cases of brain infection, anemia, hemorrhagic dropsy and mortality in the cases of heavy cardiac infection and circulatory disfunction in infection at the base of the gill lamellae respectively (Dykova *et al.*, 1986).

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In fish, the kidney is the major site of blood production. Kidney disease is one of the most puzzling fish diseases. Several myxosporean species inhabiting the kidney and urinary tract have proved to be highly pathogenic in farmed fish. The previous researchers described about the various kinds of kidney diseases. The two most important examples being the agent of proliferative kidney disease in salmonids (Clifton-Hadley *et al*., 1984; and *Sphaerospora renicola* in carp (*Cyprinus carpio*) (Dykova and Lom, 1986, 1988).

Various histopathological changes of myxosporean infected fish was described by some previous researchers. The principal pathological response is one of chronic inflammation which, in renal tissue, results in the marked proliferation of the interstitial haemopoietic tissue and a reduction in the number of glomeruli and excretory tubules. These elements are dispersed by the hyperplastic interstitial tissue, but also exhibit degenerative changes during the course of the disease. Vascular pathology, with possible haemoglobin crystallisation, has also been reported (Clifton-Hadley *et al*., 1987).

Pond fish cultures play an important role for protein food sources. In Monywa environs, there are many fish farmings and *Cirrhinus mrigala*, *Labeo rohita* and *Labeo catla* have been produced by polyculture system. Parasitic disease can be lossed to fish production. The infected fish may influence the quality of fish. Myxosporean parasites not only infect the kidneys but also the gills, the infection, clumps the gill filaments leading to the lost of gaseous exchange surface area, thus stunting the growth and resulting in the decline in the quality of fish and economic lost. Thus, there is a requisite to investigate myxosporean infection in the fishery sector. Therefore, study of myxosporean parasites in the kidneys of major carp as Cirrhinus mrigala (Hamilton , 1822) Locally Known as Nga Gyin, Labeo rohita(Hamilton , 1822) Locally Known as Nga Myint Chin and Labeo catla (Hamilton , 1822) Locally Known as Nga Thaing. *Cirrhinus mrigala*, *Labeo rohita* and *Labeo catla* was carried out from Alone fish culture Pond. The objectives of the present study are as follows:

- to investigate the prevalence of myxosporean parasites in the kidney of three carp species from Alone fish culture pond
- to examine the histological changes in the kidneys of infected fishes

Materials and Methods

This research work was conducted at the Department of Zoology,Monywa University. The study period was from June 2019 to May 2020.

Alone fish culture Pond, located in the Western part of Monywa Township (Plate 1).

Specimen collection and examination of myxosporean parasites

A total of 90 specimens of three carp species (30 specimens of each) were collected from Alone fish culture Pond, located in the Western part of Monywa Township (Plate 1).used for the investigation of myxosporean parasites. Then fish were dissected and the kidneys were removed and fixed in 10% formaldehyde solution and carried to the laboratory of Zoology Department, Monywa University. The kidneys of fishes were squashed with a drop of water on the slide by forceps and covered with a cover slip and immediately examined thoroughly for the myxosporean parasites under the microscope with digital camera image analyzer DP-12 Olympus. Fresh spores were measured according to Lom and Arthur (1989) and Lom and Dykova (1992). Morphometric measurements of fresh spores were taken in μm although lateral view of spore could not be recorded. The microphotographing was also carried out on the fresh spores.

(Source: Google Earth, 2019)

Plate 1. Map of sample collected area (Alone fish culture pond)

Identification and classification of myxosporean parasites

Myxosporean parasites were identified according to Lom and Dykova (1992), (2006), Kaur and Singh (2008), (2009), (2010), (2011), and Szekely *et al.* (2009). The spores (Myxosporean) were classified using the practical key given by Lom and Dykova (1992).

Preparation and examination for histological study

Tissue samples of kidney were studied for histological effect at tissue level. For histological study, myxosporean infected kidneys of the three carp species, *Cirrhinus mrigala*, *Labeo rohita* and *Labeo catla* were fixed in 10% formaldehyde solution and embedded in paraffin wax. Sections of 5µm thicked were cut and stained with hematoxylin and eosin. And then, myxosporean uninfected kidneys were also processed for the histological slides. All of these histological slides were examined under light microscope and photomicrographs were taken with digital camera attached to Meiji Biological Microscope (MT 4300H). The method used in this study was according to the histological method of Cambell (cited by Kolmer *et al*., 1969).

Data analysis

The prevalence rates were calculated follow after Margolis *et al*. (1982):

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fish}}{\text{Total number of fish}} \times 100$$

Results

The total 90 fish specimens of three carp species,*Cirrhinus mrigala*, *Labeo rohita*, and *Labeo catla* were studied for the prevalence of myxosporean parasites and histopathological changes in the kidney of these species. Seven species of *Myxobolus* and one species of *Thelohanellus* in *Cirrhinus mrigala*, three species of *Myxobolus* and one species of *Thelohanellus* in *Labeo rohita*, and two species of *Myxobolus* in *Labeo catla* were observed (Plate 2).

The comparison of the shape and dimensions of *Myxobolus* spp. and *Thelohanellus* spp. in three carp species is presented in Table 1. The largest spore length, 13.1 µm, was recorded in *Thelohanellus* sp. infecting *Labeo rohita* (Table 1).

The prevalence of myxosporean parasites in the kidneys of *Cirrhinus mrigala* is described in Table 2. The highest prevalence of infection was observed in *Myxobolus* sp.7, with a prevalence of 23.3%, while the lowest prevalence was found in *Thelohanellus* sp., at 10%.

The prevalence of myxosporean parasites in the kidneys of *Labeo rohita* is described in Table (3). The highest prevalence of infection was observed in *Myxobolus* sp.2, with a prevalence of 16.7%. The lowest prevalence was found in *Myxobolus* sp.7, with a rate of 6.7%, where 2 individuals were infected out of 30.

The prevalence of myxosporean parasites in the kidneys of *Labeo catla* is described in Table (4). Only two *Myxobolus* species, *Myxobolus* sp.1 and sp.2, were found in the kidneys of this fish. The prevalence of infection in *Myxobolus* sp.1 and sp.2 was 10% and 6.7%, respectively.

When comparing the prevalence of infection by myxosporean parasites in the examined fish, the highest prevalence was found in *Cirrhinus mrigala* at 83.3%, with 25 out of 30 individuals infected (Table 5).

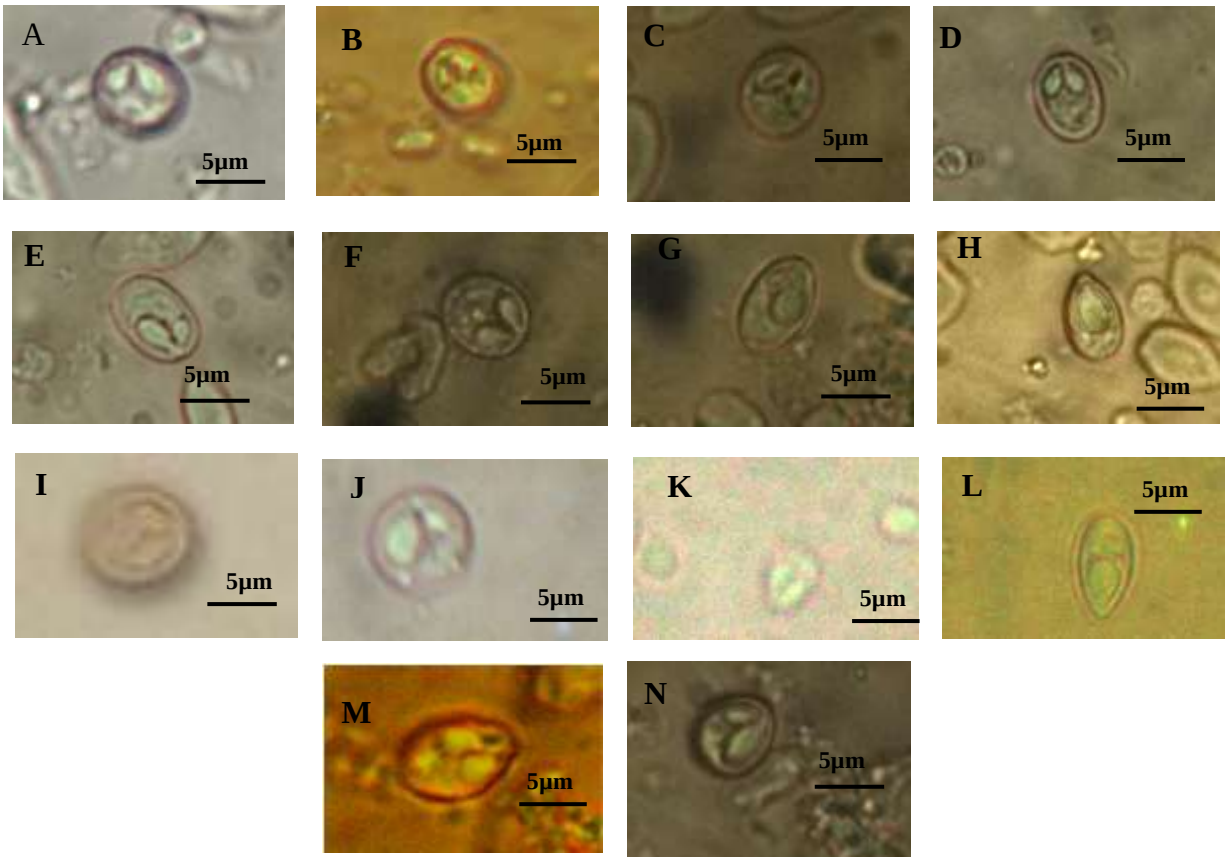

Plate 2 Myxosporean parasites found in the kidney of three carp species (A to H myxosporean parasites of *Cirrhinus mrigala*; I to L myxosporean parasites of *Labeo rohita*; M and N myxosporean parasites of *Labeo catla*)

Table 1 Comparison of shape and dimension of *Myxobolus* spp. and *Thelohanellus* sp. of three carp species (measurements are described in µm)

Sr. no.	Parasite	Host	Infected organ	Spore shape	Spore length	Spore width	Large		Small	
							Polar capsule length	Polar capsule width	Polar capsule length	Polar capsule width
1	*Myxobolus* sp. 1	*Cirrhinus mrigala*	Kidney	Rounded	8.33	8.33	5.00	3.33	3.33	2.22
2	*Myxobolus* sp. 2	*Cirrhinus mrigala*	Kidney	Pyriform	11.87	7.33	6.15	3.69	3.69	2.46
3	*Myxobolus* sp. 3	*Cirrhinus mrigala*	Kidney	Ovoidal	8.7	6.8	4.44	1.66		
4	*Myxobolus* sp. 4	*Cirrhinus mrigala*	Kidney	Subspherical	11.67	8.34	4.44	2.222		
5	*Myxobolus* sp. 5	*Cirrhinus mrigala*	Kidney	Elongated	12.22	8.33	6.11	3.33	3.33	2.22
6	*Myxobolus* sp. 6	*Cirrhinus mrigala*	Kidney	Spherical	11.11	10.0	5.0	2.77		
7	*Myxobolus* sp. 7	*Cirrhinus mrigala*	Kidney	Ellipsoidal	10.55	7.33	5.55	3.33	2.77	1.66
8	*Thelohanellus* sp.	*Cirrhinus mrigala*	Kidney	Tear- shaped	11.11	7.22	6.66	5.00		
9	*Myxobolus* sp. 1	*Labeo rohita*	Kidney	Subspherical	10.17	5.82	3.75	2.5		
10	*Myxobolus* sp. 2	*Labeo rohita*	Kidney	Subspherical	10.6	8.75	5.0	3.7	2.5	1.25
11	*Myxobolus* sp. 3	*Labeo rohita*	Kidney	Pear shaped	7.5	3.75	3.75	1.7		
12	*Thelohanellus* sp.	*Labeo rohita*	Kidney	Tear-drop shaped	13.1	6.8	6.25	3.7		
13	*Myxobolus* sp. 1	*Labeo catla*	Kidney	Ovoidal	4.92	3.69	1.85	1.54		
14	*Myxobolus* sp. 2	*Labeo catla*	Kidney	Ovoidal	7.38	4.92	3.69	2.46	2.46	1.85

Table 2 Prevalence of myxosporean parasites in the kidney of *Cirrhinus mrigala*

Sr. no.	Parasite	Examined fish	Infected fish	Prevalence (%)
1	*Myxobolus* sp.1	30	6	20
2	*Myxobolus* sp.2	30	6	20
3	*Myxobolus* sp.3	30	4	13.33
4	*Myxobolus* sp.4	30	4	13.33
5	*Myxobolus* sp.5	30	5	16.67
6	*Myxobolus* sp.6	30	5	16.67
7	*Myxobolus* sp.7	30	7	23.33
8	*Thelohanellus* sp.	30	3	10

Table 3 Prevalence of myxosporean parasites in the kidney of *Labeo rohita*

Sr. no.	Parasite	Examined fish	Infected fish	Prevalence (%)
1	*Myxobolus* sp.1	30	6	20
2	*Myxobolus* sp.2	30	5	16.67
3	*Myxobolus* sp.3	30	2	6.67
4	*Thelohanellus* sp.	30	6	20

Table 4 Prevalence of myxosporean parasites in the kidney of *Labeo catla*

Sr. no.	Parasite	Examined fish	Infected fish	Prevalence (%)
1	*Myxobolus* sp.1	30	3	10
2	*Myxobolus* sp.2	30	2	6.67

Table 5 Total prevalence of myxosporean parasites from two carp species

Sr. no.	Host	Examined fish	Infected fish	Prevalence (%)
1	*Cirrhinus mrigala*	30	25	83.33
2	*Labeo rohita*	30	16	53.33
3	*Labeo catla*	30	4	13.33

Histopathological effects in the Kidneys of three carp species

In *Cirrhinus mrigala*, any changes were not occurred in the uninfected normal kidney (Plate 3 A). However, in serious infected fish, necrotic lesions were more distinct among the renal tubules (Plate 3 B). Some glomeruli were cluster, shrinkage and atrophy (Plate 3 C). The spores were accumulated and dispersed in the kidney tubules (Plate 3 D). The mature myxosporean parasites liberated from the plasmodium were observed (Plate 3 E).

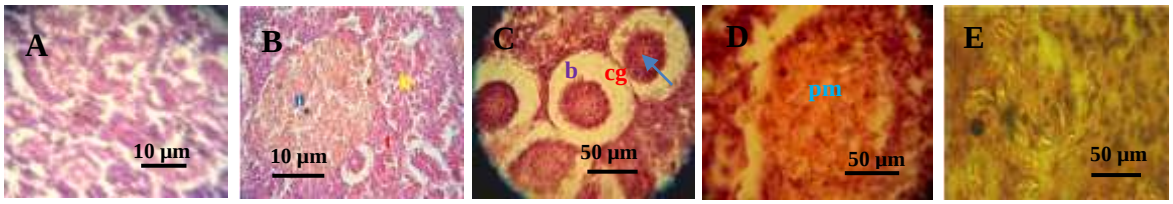

Plate 3 Histological changes in the Kidneys of *Cirrhinus mrigala*

In *Labeo rohita*, the kidneys infected with myxosporean parasites showed that the renal tubules were shrinkage and lumen was occlusion (Plate 4 A). The glomerulus was shrunk into atrophy (Plate 4 B). The accumulation of inflammatory cell was observed among the renal tubules (Plate 4 C). In serious infected kidney, the large amount of mature myxosporean parasites was observed in the epithelial cells of renal tubules, in the blood corpuscle of kidney tissue and glomerulus (Plate 4 F). In *Labeo catla*,the kidney tubules were Necrosis and distortion (Plate 5 A), the glomerulus were atrophy of and necrosis found around the glomerulus (Plate b B), the glomerulus were Fusion (Plate 5 C) and the lumen in the renal tubule were degeneration and closing (Plate 5 D).

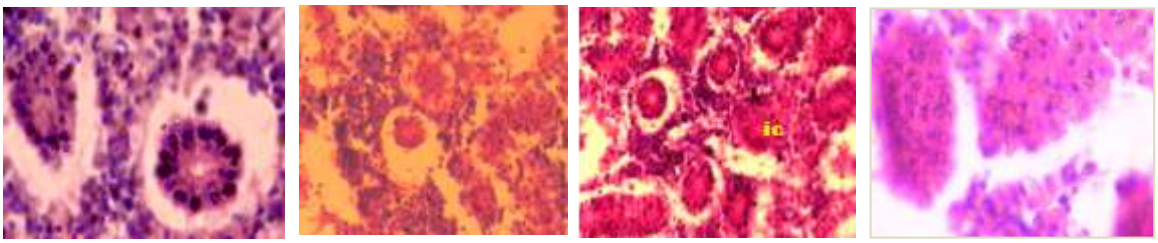

Plate 4 Histopathological changes in the kidney of *Labeo rohita* by myxosporean parasites

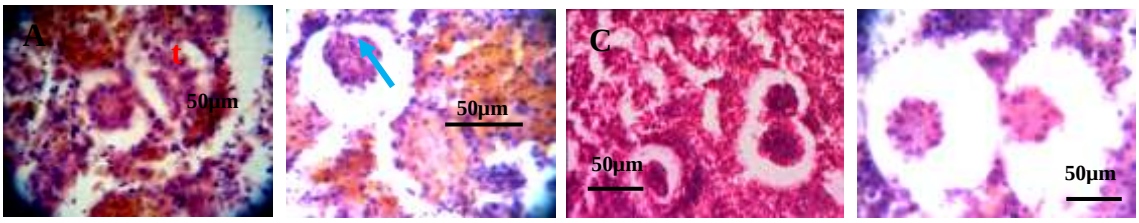

Plate 5 Histopathological changes in the kidney of *Labeo catla* by myxosporean parasites

Discussion

Comparison of myxosporean parasites from polyculture carp species, *Labeo rohita*, *Cirrhinus mrigala* and *Labeo catla* were studied. The total of 90 specimens (30 specimens in each fish species) was examined. In *Cirrhinus mrigala* eight species of myxosporean parasites (seven *Myxobolus* spp. and one *Thelohanellus* sp.) were occurred. In *Labeo rohita*, four species of myxosporean parasites (three *Myxobolus* spp. and one *Thelohanellus* sp.) were found. In *Labeo catla*, two species of myxosporean parasites (two *Myxobolus* spp.) were observed. But the shapes and dimensions of spores were not similar between these species. It is concluded that *Cirrhinus mrigala* more diversed species of myxosporean parasites and more infected than the others two carps. But the polyculture fish host, different myxosporean parasites were observed in the present study.

In *Cirrhinus mrigala*, of prevalence of *Myxobolus* sp.1, 20 %, *Myxobolus* sp.2, 20 %, *Myxobolus* sp.3, 13.33 %, *Myxobolus* sp.4, 13.33 %, *Myxobolus* sp.5, 16.67 %, *Myxobolus* sp.6, 16.67 %, *Myxobolus* sp.7, 23.33 % and *Thelohanellus* sp. 10 % were observed. In *Labeo rohita*, *Myxobolus* sp.1, 20 %, *Myxobolus* sp.2, 16.67 %, *Mxobolus* sp.3, 6.67 % and *Thelohanellus* sp., 20 % were found. In *Labeo catla*, *Myxobolus* sp.1, 10 % and *Myxobolus* sp.2, 6.67 % were observed.

The prevalence of *Myxobolus* sp.7 (23.33 %) was predominant agent in *Cirrhinus mrigala. Myxobolus* sp.1 and *Thelohanellus* sp. (20 %) were predominant agent in *Labeo rohita* and *Myxobolus* sp.1 . (10 %) was predominant agent in *Labeo catla* during the present study. Lom and Dykova (1992) stated that all functions of the kidney are fluid balance, waste excretion and blood cell production become inexorably compromised and the glomerulus is the main component of the renal corpuscle and it composed of blood capillary loops. The blood is selectively filtered as it flows through the glomerular capillaries.

Pa Pa Win (2007) reported that the histological changes in the kidney of *Cirrhinus mrigala*, the mature spores of *Myxobolus* spp. are attached to the lumen of renal tubule and convoluted tubule of kidney. In the kidney of host fish heavily infected plasmodia containing mature spores of myxosporean parasites destroyed the glomerulus and interstitial cells. Early developmental stage of myxosporean parasites were also observed in kidney interstitial cell and glomerulus. In kidneys of host fish, numerous spores and large plasmodium of myxosporean parasites were not found. In the present study, the observations of histological changes in the kidney of *Cirrhinus mrigala* were

agreed with Pa Pa Win (2007). But in the present study, numerous spores were distributed and large plasmodium of myxosporean parasites also observed.

Moe Kyi Han (2006) reported that the histological changes in the kidneys of *Labeo rohita* were early developmental stages of parasites, damages in glomerulus and renal tubules, developmental stages of myxosporean parasites were found in the lumen of renal tubules. In the present study, the histological changes in the kidneys of *Labeo rohita* were observed as deformities; dilation of glomerulus, shrinkage of collecting duct, degeneration of lumen in the renal tubules, hypertrophy and hyperplasia of glomerular cells, swelling of glomerular epithelial cells and *Myxobolus* sp. was found in the blood corpuscles.

Moser and Kent (1994) recorded that affected myxosporean parasites in *Parvicapsule* sp. (coho salmon) are dark and lethargic and postmortem examination reveals renal hypertrophy and hemorrhage beneath the renal corpusles. Spores and other developmental stages occupy the epithelium and lumina of the renal tubules. The infection is associated with severe dilation and necrosis of the renal tubules. In the present study, the histological changes in the kidneys of *Labeo catla* were as cluster and atrophy of glomerulus, fusion of glomerulus more occurred.

The myxosporean infected kidneys of *Cirrhinus mrigala* and *Labeo rohita* the distortion were more occurred and the parasite spores were more incident than *Labeo catla*. Large necrotic lesions, spores and other developmental stages occupy the epithelium and lumina of the renal tubules were found in the kidney tissue of all infected fish hosts.

Bruno *et al*. (2006) described that myxosporidian infections can cause all categories of regressive and progressive pathological changes in the host including atrophy, dystrophy, hypertrophy, hyperplasia, necrosis and inflammation. Similarily most myxosporidian species cause minimal tissue damage, relatively few species are known to cause serious or fatal infections. Pathological changes similar to severe cases, led to nephrosis and necrosis of the tubular epithelium. Desser *et al*. (1983) and Supamattaya *et al*. (1991) also noted vacuolation of renal tubule epithelial cells in fish infected with myxosporean parasites, *Sphaerospora angulata* and *S. epinepheli* respectively.In the present study, myxosporean parasites were found within the blood corpuscles of kidney tissues.

The three studied fishes were cultured species and of economic importance. The enhancements of fish production are depended on healthily growth fish; there is need for adequate knowledge of parasites that infect them. The present described species of 14 myxosporean parasites were different in size and shape. The mix myxosporean infected fish was mostly found in fish culture pond. Among the three carp species, *Cirrhinus mrigala* was the most infected mix myxosporean parasites in fish culture pond. The fish parasites and diseases are the most important problem due to decrease fish production and death. The myxosporean parasites are one of the most important in fish. These parasites were incident in all parts of the body of fish host. Therefore, this research is needed to carry out for studying myxosporean parasites as well as disease of fish.

In conclusion, three carp species of fish hosts from Alone fish culture Pond, Monywa Township were parasitized by 14 species of myxosporean parasites. The fish hosts could be threated to their health by these parasites and would lead a condition to loss of the fish population. It should carefully consider managing fish health, to control or reduce measure for the parasites infection and to conserve fish fauna. The information in this work is expected to be useful for local consumers and further researchers.

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