Title	Histological Study on the Myxosporean Infection in the Gills of Common Carp, Cyprinus carpio (Linnaeus,1785)
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Histological Study on the Myxosporean Infection in the Gills of Common Carp, *Cyprinus carpio* (Linnaeus, 1785)

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Abstract

In the histological study, mature spores of *Myxobolus* sp. 2 were found at the tip of the gill filament of *Cyprinus carpio*. Two types of plasmodia were found and were rounded in shape. Small plasmodia (mean: L = 36.8 x W = 36.5 μm) were observed between gill lamellae. Large plasmodia (mean: L = 150.4 x W = 106.0 μm) were also found in the distal, middle and basal region of the gill filament. Plasmodia were found along the length of the gill filament. Some plasmodia were also found in the gill lamellae. Dilation of blood vessels in the gill filament and infiltrated cells were found as histopathological changes. With the evidence of histological changes, myxosporean parasites may cause a disturbance in the respiratory process of the infected common carp.

Key word: *Myxobolus* sp., *Cyprinus carpio*, plasmodia, myxosporean,

Introduction

Aquaculture plays an important role in Myanmar economy. It is known that 25 % of total fish production was produced by freshwater fisheries. Fourteen species of freshwater fish are known to cultivate extensively throughout the country. Among these species, *Cyprinus carpio* also is of economic importance in Myanmar. On the other hand, parasites and diseases are one of the major problems in fish especially cultured in restricted body of water since pathogens can easily spread among the fish confined to the limited one(Hla Win, 2004).

Among the parasites of fish, myxosporea is a large group of parasites causing serious damage to the aquaculture industry. There are approximately 1350 species of myxosporean distributed in 52 genera, most of which parasitize freshwater fish (Kent *et al*., 2001). Economically important diseases caused by myxosporean parasites were swim bladder inflammation (SBI), proliferative kidney disease (PKD), whirling disease (WD) and proliferative gill disease (PGD). It is also recorded that *Myxobolus longisporus* infected the gill of *Cyprinus carpio* in some Chinese lakes and the cyst-like plasmodia are localized in the secondary lamellae (Dykova *et al*., 2003). Similarly, the infection of *Myxobolus* sp. in the gills of the common carp was reported by Yokoyama *et al*. (1997a) and two types of *Myxobolus* cysts were recognized. One was a harmless small

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cyst. Moreover, infection of *Myxobolus artus* in the skeletal muscle of carp, *Cyprinus carpio* was also reported (Ogawa *et al*., 1992).

Molnar (2002) proposed six types of plasmodia according to the site of manifestation of the plasmodia. The most common types of plasmodia were interlamellar type, intralamellar type, filamental vascular type, filamental epithelial type, filamental intrachondreal type and basifilamental type.

Based on the above references it can be clearly seen that myxosporean parasites infect both fresh and marine fishes, under cultivation in the ponds as well as in the wild. Thus, it became important to study the biology of the causative agent of the disease and to find the ways to control the spread of pathogen. In the present work, the common carp *Cyprinus carpio*, was chosen for the histologically investigation of the parasites infection.

The objectives of the present study are:

1) to confirm the location of parasite in gill of fish host
2) to study the histological changes induced by myxosporean infection in the gill of fish host.

Materials and Methods

Fish were collected from Thayetkone fish farm. Gills were cut from each specimen and were examined for the incidence of myxosporean parasite infection using wet mount method under the light microscope. For histological studies, the gill of 14 infected specimens were examined. The infected gills were fixed in 10% formalin, and processed routine histological procedures, sectioned at 3 μ and stained in haematoxylin and eosin. Similarly, gill from the 7 uninfected fish were also processed for control slides. Then, the histological slides were studied under Olympus light microscope and photomicrographs were taken with DP 12 digital camera. According to the size of plasmodia, small plasmodia were determined as the size of length and width, < 100.0 μm, and those of large plasmodia were > 100.0 μm, respectively. Two slides with 4 to 5 section for each infected fish.

Results

The infection caused clubbing of gill lamellar, pushing aside the neighbouring gill lamellae (Fig.4). Fusion of gill lamellae resulted from proliferative inflammatory cell infiltrations (Fig.2) and epithelial hyperplasia (Fig.3). The histological change most commonly observed was dilation of blood vessel in all (100 %), followed by hyperplasia (92.8 %), epithelial cells separation (64.2 %), damage of blood vessel (64.2 %), inflammation cell infiltration (64.2 %) and lamellae shortened (57.1 %). The moderately changes observed were 42.8 % for lamellae fusion, followed clubbing (42.8 %), and plasmodia (28.5 %) with plasmodia, respectively (Table.2).

Mature spores of *Myxobolus* sp. 2 were also found in the gill filament (Fig.5). Moreover, small plasmodia (mean: L = 36.8 x W = 36.5 μm) were seen (Table.1) with rounded or irregular shape between gill lamellae (Fig.6). In some fish, plasmodia occupied the length of the gill filament which were deformed and distended (Fig.7). Then, large plasmodia (mean: L = 150.4 x W = 106.0 μm) were located at the tip, middle and base of the gill filament of infected fish (Fig.8 and 9).

Histological changes in the gill

In the infected fish, mature spores of *Myxobolus* sp. 2 were found in gill filament (Fig.5). These spores were easily distinguishable by their shape. Many small plasmodia (mean: L = 36.8 x W = 36.5 μm) occupied the length of the gill filament and between gill lamellae. Some large plasmodia (mean: L = 150.4 x W = 106.0 μm) were also found at the tip, middle and basal region of the gill filament.

The infection caused destruction and distiguration of the lamellae, fused together and touched to each other. Normal structure of lamellae was lost and severe hyperplasia in epithelial cells was observed (Fig.14). In some infected gill, blood cells released from blood vessel in gill lamellae (Fig.15), shortening of gill lamellae (Fig.16) and dilation of blood vessel were observed (Fig.17). In some fish, dilation of blood vessels associated with seperation of gill filament was found (Fig.18). Such characters were mostly observed in infected fish but not in control (uninfected) fish (Fig.1).

Infection associated with lamellae

Interlamellar type (Small plasmodia, mean: L = 36.8 x W = 36.5 μm)

In this type of infection (Fig.2) plasmodia were mostly found in the basal region of stratified epithelium between the gill lamellae. Some plasmodia located at the tip of gill lamella (Fig.10).

Filamental type (Large plasmodia, mean: L = 150.4 x W = 106.0 μm)

Mature spores of *Myxobolus* sp. 2 were observed at the tip of the gill filament (Fig.5). Large plasmodia were also found at the tip, middle and base of the gill filament of fish (Fig.8 and 9). Then Small plasmodia were also found at the tip of gill filament (Fig.11).

Intrachondreal type (Large plasmodia, mean: L = 150.4 x W = 106.0 μm)

This type was observed in the cells of the cartilaginous supporting tissue of the gill filaments. Moreover, plasmodia were formed in blood vessel in middle region of gill filament (Fig.12).

Basifilamental type (Large plasmodium, mean: L = 150.4 x W = 106.0 μm)

The plasmodium of the basifilamental type was observed in the epithelial tissue between the gill filaments, on the surface of the gill arch (Fig.13).

Discussion

In the present work, out of six types of plasmodia only four types, namely interlamellar type, filamental type, intrachondreal type and basifilamental type were observed. Blood vessels infected by the parasite become enlarged, some damaged with the release of blood cells the red blood cells into the gill lamellae. Dilation of blood vessels resulted in complete loss of normal structure in some infected fish. Moreover, large plasmodia at the tip of some gill lamella resulted in club-shaped structure.

With reference to the site preference of fish myxosporeans in the gills, four types of plasmodia were classified by Molnar (2002). The four types are: Type I. plasmodia in the gill lamellae, which included small cysts infecting the interlamellar epithelium and large one, producing deformities in several gill lamellae; Type II. plasmodia located in the gill filament,

some of which found in the intrafilamental epithelium; Type III. are those which occupied the base of the gill filaments known as basifilamental plasmodia; Type IV. plasmodia are located in the gill arch.

Two types of plasmodia, large and small types of plasmodia, were recognized. Although it was disclosed that the large type plasmodia developed along the primary lamella and small plasmodia grew in the secondary lamella in the gills of the common carp (Yokoyama *et al*., 1997). The histology of the gill in the present work showed that the large plasmodia were located at the tip of the gill filament and also at the base as well as in the middle of blood vessel, while the small plasmodia occupied along the length of the gill filament. Both types of plasmodia were also found to infect the same lamella of the gill filament (Fig.19).

Among the 14 infected fish examined (Table.2), the dilation of blood vessels was revealed in all the samples. Hyperplasia was observed in 92.8 % of the infected fish (n = 13), accompanied by epithelial cells separation of the gill tissues 64.2 % (n = 9) and damage of blood vessel 64.2 % (n = 9). Infiltrated cells especially white blood cells were observed in the tissues 64.2 % (n = 9) of the infected fish. It is therefore assumed that such a very high percentage of histopathological changes in the host would probably harm the animals concerned, since gills are the primary organs for respiration and which exchange of gas take place.

It is difficult to conclude whether the two types of plasmodia belong to the same species of parasites or not. However morphometrical comparison between the plasmodia revealed a distinct difference in size (Table 1).

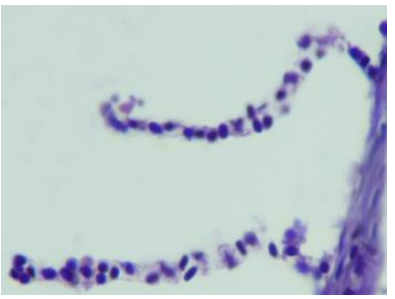

Fig. 1 Normal structure of gill (x1000)

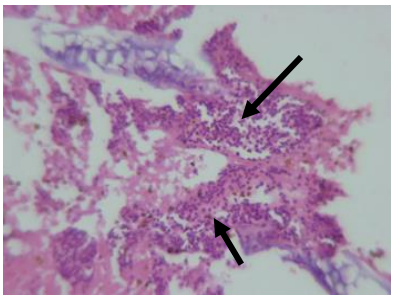

Fig. 2 Inflammatory cells infiltration in gill filament (arrow) (x400)

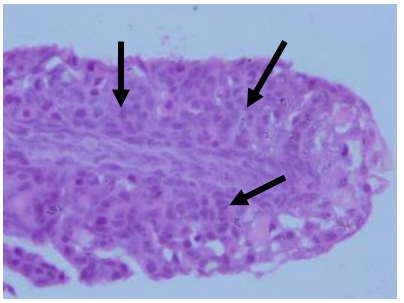

Fig. 3 Hyperplasia (arrows) in gill lamellae (X1000)

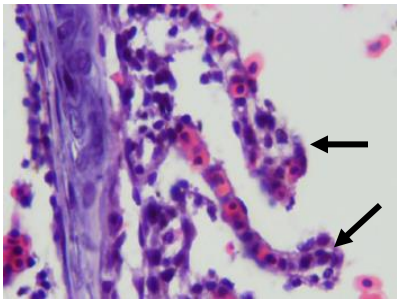

Fig. 4 Swelling (arrows) of lamellae into club-shaped structure (x400)

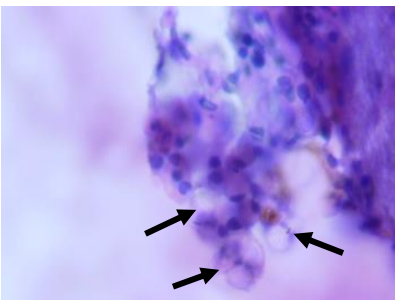

Fig. 5 Mature spores (arrows) of *Myxobolus* sp. 2 in the gill filament (x400)

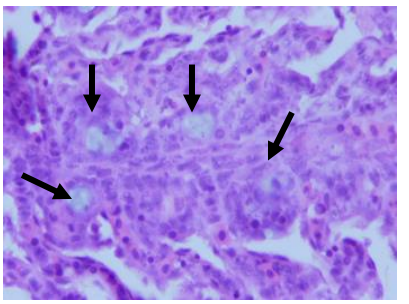

Fig. 6 Small plasmodia (arrows) between gill lamellae (x 400)

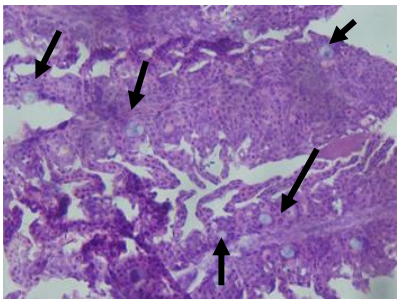

Fig. 7 Small plasmodia (arrows) in the length of gill lamellae (x 400)

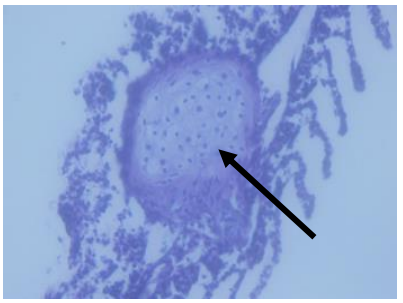

Fig. 8 Large plasmodia(arrow) at the tip of gill filament(x400)

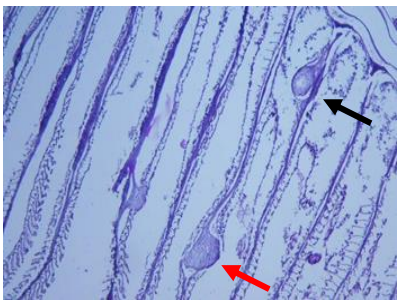

Fig. 9 Large plasmodia in the middle (red arrow) and base (black arrow) of gill filament(x400)

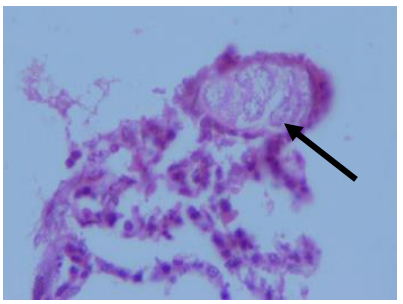

Fig. 10 Plasmodium (arrow) at the tip of gill lamellae(x400)

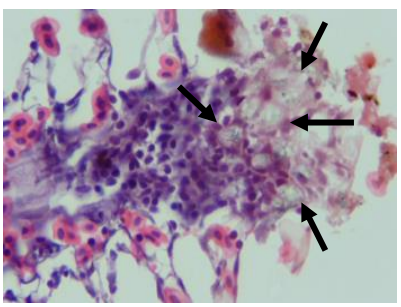

Fig. 11 Small plasmodium (arrow) in the gill filament (x 1000)

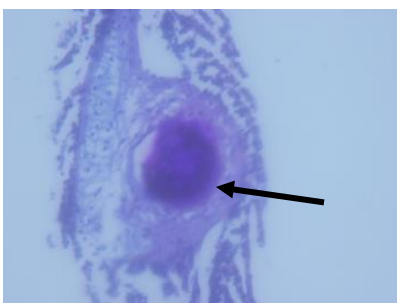

Fig.12 Large plasmodium (arrow)in the middle region of blood vessel (x400)

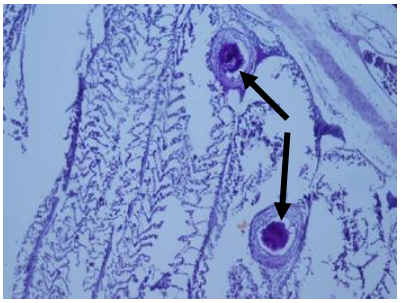

Fig.13 Large plasmodia (arrows) at the base of gill filament (x 400)

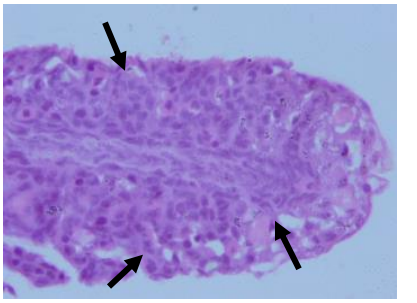

Fig.14 Hyperplasia and loss of nomal appearence of gill lamellae (x 400)

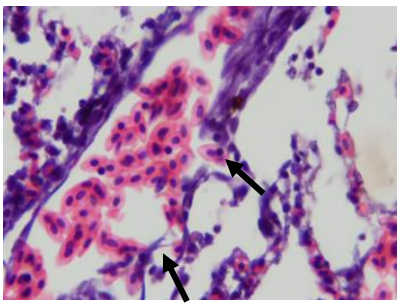

Fig.15 Blood cells release (arrows) from blood vessel (x1000)

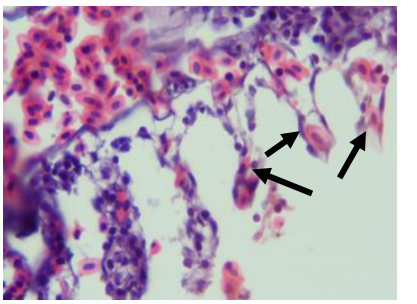

Fig.16 Shortening (arrows) of gill lamellae (1000)

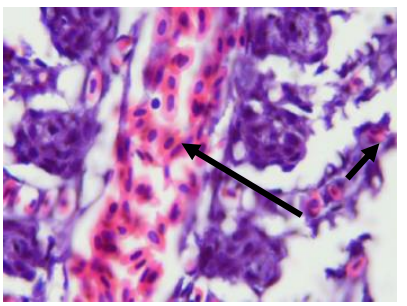

Fig.17 Dilation (arrow) of blood vessel in gill filament (x1000)

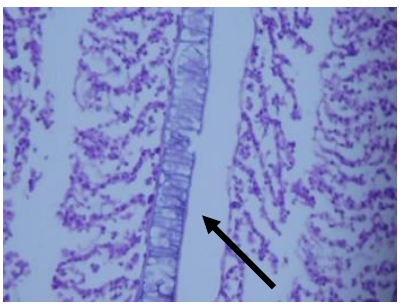

Fig.18 Epithelial cells separation (arrow) of gill lamella (x400)

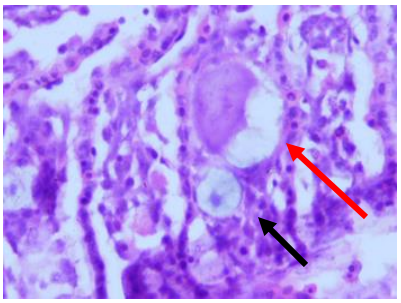

Fig.19 Small (black arrow) and large (red arrow) plasmodia between gill lamellae (x 400)

Table . 1 Comparison of large and small plasmodia of *Cyprinus carpio*

	Large plasmodia (N=9)		**Small plasmodia (N=20)**	
	Length (μ)	**Width (μ)**	**Length (μ)**	**Width (μ)**
Average	170.8	125.7	36.32	31.95
Range	129.2 – 233.7	104.5 – 147.6	19.7 - 73.8	19.7 – 79.9

N = numbers of plasmodia

Table 2. Histological changes in the infected gill

Fish No.	Spore	Plasmodia	Hyperplasia	Epithelial cells separation	Blood vessel dilation	Blood vessel damaged	Lamellae fusion	Lamellar clubbing	Lamellae shortening	Infiltrated cell
1	-	-	+++	-	++	-	-	-	-	++
2	-	++ *	+	++	+	-	-	++	++	+
3	-	++ *	+	++	+	+	-	+	+	+
4	+	-	+++	+	+++	+++	-	+++	-	++
5	-	+++ **	+++	++	++	-	++	-	+	+
6	-	-	++	++	++	+	++	-	+	-
7	-	-	++	+	++	+	+	+	+	+
8	-	+ *	+++	++	++	-	-	-	+	+
9	-	-	+++	++	++	++	++	++	+	+
10	-	-	++	-	+	+	-	-	-	++
11	-	-	++	-	+	+	+	-	-	-
12	-	-	-	+	+	+	-	-	+	-
13	-	-	++	-	+	-	-	-	-	-
14	-	-	++	-	++	+	+	+	-	-
Total	1/14 (7.1%)	4/14 (28.1%)	13/14 (92.8%)	9/14 (64.2%)	14/14 (100%)	9/14 (64.2%)	6/14 (42.8%)	6/14 (42.8%)	8/14 (57.1%)	9/14 (64.2%)

+Low,++ medium and+++ high were determined according to the occurrence on each slide.

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