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MYXOZOSPOREAN INFECTION IN THE MUSCLES OF *CIRRHINUS MRIGALA* (HAMILTON, 1822)

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Abstract

The two types of *Myxobolus* species were recorded in the muscles of *Cirrhinus mrigala* collected from Yezin Fisheries Station, Nay Pyi Taw. The plasmodia of *Myxobolus* sp. containing with mature spores in the central part of the plasmodia were bounded by single-unit membrane in contact with the muscle tissues. Spores of *Myxobolus rohitae*, measured 11.6µm ± 1.8µm in length and 9.1µm ± 0.9µm in width and appeared elongated ellipsoidal in valvular view. Polar capsules were pyriform, equal and sporoplasm granular present. Spores of *Myxobolus* sp., measured 11.6µm ± 1.1µm in length and 7.6µm ± 0.8µm in width in valvular view. Two polar capsules were slightly pyriform and unequal in shape, larger 5.0µm ± 1.1µmx3.3µm ± 0.5µm and smaller 3.5µm ± 0.8µmx3.3µm ± 0.5µm in size. The highest prevalence of *Myxobolus rohitae* infection (48%) was recorded in July, 2019 with the highest mean intensity 2.5. The prevalence of *Myxobolus* sp. infection was highest 62% in August with the highest mean intensity 3. Histopathological changes such as loss of epidermis, dermis split and separated from muscle and hemorrhage were found in muscle layer. The present study represents the first report of *Myxobolus* infection in the muscle tissues of *Cirrhinus mrigala* in Myanmar.

Introduction

Fishery and aquaculture has an important role in the local economy in Myanmar. Fish diseases may cause severe losses of fish farms through reduced fish growth and production. The parasites may involve in the serious outbreak of disease in fish farms (Kayis *et al*., 2009). It is a major problem that carrying heavy infestation of parasites of freshwater fishes in aquaculture. They have been receiving considerable scientific attention due to serious damage to fisheries resources by them (Ravichandram *et al*., 2009). Infectious diseases of cultured freshwater carps are one of the major problems to successful aquaculture industry. The outbreak of various types of disease is one of the important reasons of reduction in the fish production. Therefore, proper health management procedures should be followed with appropriate control measures to boost up aquaculture production. In the high stocking condition, particularly if the fishes are stressed, the parasites multiply rapidly.

Parasites of the phylum Cnidarian have been described in lower vertebrate hosts, mainly in fish and in some amphibians. They are the most common fish parasites, infecting both marine and freshwater fish (Eiras *et al*., 2005). The genus *Myxobolus* is the richest group among Myxosporidia, containing about 744 nominal species (Eiras *et al*., 2005). The ability of some Myxosporea species to transmit as a barrier between fish and humans (Boreham *et al*., 1998; Moncada *et al*., 2001) does not prevent the possibility that the *Myxobolus* sp. may also be transmitted to humans. The zoonotic potential of *Myxobolus* sp. cannot be ignored. Infection in humans is associated with the consumption of raw and undercook fish containing live parasites. The *Cirrhunus mrigala* has been an important commercial fish species in Myanmar for a long time. However, parasitic diseases are a significant restricts for the development of the carps culture industry, among which especially myxobolosis, which has become one of the most notably growing parasitic infections. The genus *Myxobolus* was first established by having spores with or without an iodinophilous vacuole and with one or two polar capsules (Butschli, 1882). This genus has the global distribution and highest number of Myxosporidia.

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The infection by *Myxobolus* sp. is characterized by the formation of cysts in the tissues of fish with mature spores. The presence of these cysts has been associated with tissue lesions, gross deformities, and organ malfunction (Feist 2008). Myxospores have been incidentally found in human fecal samples, which were collected to investigate the intestinal diseases caused by protozoa of medical importance (Bradbury *et al*. 2015). The showing of myxospores in human feces associated with ingestion of contaminated food or undercooked fish (Kawai *et al*., 2012). However, Japanese food types, like sushi and sashimi are now consumed worldwide. This has resulted in an increase of food-borne diseases caused by the ingestion of raw fish (Barralet *et al*., 2004).

Muscle is the main component of the fish body. It is a common site of establishment for various myxosporeans in infecting fishes. In a synopsis of *Myxobolus* species of the world, 54 of the 751 characterized species have been described from the muscles (Eiras *et al*., 2005). In another synopsis on *Henneguya* species, 6 of the recorded 146 species were found to infect muscles (Eiras 2002). In addition, muscle cells contain blood vessels, nerves and connective tissue as well as cartilaginous and bony elements (Ferguson 1989). In several fish, myosepta are supported by bony portions formed by a dense connective tissue which has been calcified in some fish. Therefore, due to their different tissue affinities, myxosporeans can occur in different locations and might affect muscle cells, connective tissue, bones and blood vessels. *Myxobolus sandrae* infected to the intermuscular connective tissue in the pike-perch, *Sander lucioperca* was observed (Molnar and Szekely, 2014). Examination of parasitic infections in muscles of *Cirrhinus mrigala* in Myanmar is still required to improve production. The present study was therefore undertaken to detect the myxozoan parasites infected to the muscles of *Cirrhinus mrigala* in Yezin fishery station, one of the biggest *C. mrigala* hatchery in Myanmar and to evaluate the histopathological alterations caused by Myxosporean parasitic infestation in muscles of *Cirrhinus mrigala*.

Materials and Methods

Study Area

Yezin Fishery Station is a government owned fish seed multiplication center. It is situated at 19° 50' 14.9" N and 96° 16' 36.8" E about 19 km away from Pyinmana city, and it is located near the Yezin Dam. It is one of the biggest *Cirrhinus mrigala* hatcheries in Myanmar, and also distributes fry/fingerling *C. mrigala* through the country.

Study Period

The research work was carried out from August 2018 to September 2019.

Sample Collection and Examination of Parasites

Cirrhinus mrigala fingerlings were cultured in experimental pond (8.3mx33.3m) at Yezin Fishery Station as extensive culture system. Thirty fish were collected monthly to examine the occurrence of parasites. A total of 30 fish samples were carried to the laboratory of Department of Aquaculture and Aquatic Diseases, University of Veterinary Science or laboratory of Aquatic Bioscience, University of Yangon with oxygen filled plastic bags. The total length, standard length and body weight of each specimen were immediately measured and recorded. Fish were dissected and muscle tissues were collected to examine the parasites. The muscle tissues were used for smear slide preparation and histological slides preparation. For smear slide preparation, the muscle tissues of fish were checked under stereomicroscope for the cyst formation of myxosporean. Muscles were squeezed with cover slip with 1 drop of normal saline (0.9% NaCl). Occurrence of parasites was examined under light microscope, Olympus – CX 31.

Identification of parasites

Identification of myxosporean parasites was conducted on the various morphological structures of spore including shape, size, number of polar capsules, length and number of coils of polar filaments, intercapsular process presence or not, number of nuclei and iodinophilous vacuole in the sporoplasm, etc. according to the guidelines of Lom and Dykova (1992) and Kalavati and Nandi (2007). They were measured and photographed using the light microscope (Olympus CX 31) under x100 magnification.

Data analysis for parasites

Prevalence of parasitic infection was calculated in accordance with the following methods (Bush *et al*., 1997).

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

Mean intensity of infection was classified four stages according to Culloty *et al*. (1999).

Stage (I): 1-20 parasites observed within five minutes of screening under x40 magnification

Stage (II): 21-40 parasites observed within five minutes of screening under x40 magnification

Stage (III): 41-60 parasites observed within five minutes of screening under x40 magnification

Stage (IV): 1-10 parasites in all field of region observed immediately in screening under x40 magnification

$$\text{Mean Intensity} = \frac{\text{Total Number of parasites recovered}}{\text{Total number of infected fishes}}$$

Preparation of Histopathological Slides

To understand the histological changes of infested tissues of muscles, and infected tissue with cyst formation were fixed with 10% neutral buffered formalin. After fixation for 48 hours, the tissues were cut in order to obtain a size of 1 cm^3. The prepared tissues were dehydrated through a graded series of ethanol, cleared in xylene, and infiltrated in the paraffin. Sections were cut at 5µm in thickness on a microtome (TBS SHUR/Cut 2500) fitted with a sharpened microtome knife. These sections were then stained with Hematoxylin-Eosin. The permanent mounting of the slides was made by DPX (distyrene, plasticizer and xylene). Histopathological lesions were examined and photographed at different magnifications with the help of binocular microscope with digital camera and attached monitor (Olympus – CX 31).

Results

Myxobolus spp. infection in the muscles of *Cirrhinus mrigala*

Myxobolus rohitae and *Myxobolus* species were recorded in the muscles of *Cirrhinus mrigala* collected from Yezin Fisheries Station. *Myxobolus* sp. was identified according to Lom and Arthur (1989), Lom and Dykova (1992) and Kalavati and Nandi (2007).

Plasmodia

The plasmodia of *Myxobolus* sp. were bounded by single-unit membrane in contact with the muscle tissues of the host (Plate 1, A and Plate 2, A). The central part of the plasmodia contained the mature spores.

Morphometry of *Myxobolus rohitae*

Spores are roughly oval to elongate ellipsoid in front view, sometimes with a semicircular ledge or mucus envelope at the posterior end. Anterior end of the spore is extremity pointed and posterior end is rounded. Spores of *Myxobolus rohitae*, measured 11.6µm±1.8µm in length and 9.1µm±0.9µm in width and appeared elongated ellipsoidal in valvular view. Polar capsules were pyriform, equal and polar filament well marked inside the capsule in fresh spores, sporoplasm granular present. Length and width of polar capsules were 3.2µm±0.4µmx2.3µm±0.4µm in size (Plate 1).

Morphometry of *Myxobolus* species

Spores of *Myxobolus* sp., measured 11.6µm±1.1µm in length and 7.6µm±0.8µm in width and appeared elongated ellipsoidal in valvular view. Two polar capsules were slightly pyriform and unequal in shape with 4 to 6 filaments, larger 5.0µm±1.1µmx3.3µm±0.5µm and smaller 3.5µm±0.8µmx3.3µm±0.5µm in size (Plate 2). Sporoplasm was finely granular and occupied most of the extracapsular cavity of spore. Spores were elongated and ellipsoid in valvular view with mucus envelope around the posterior end. Two polar capsules were slightly pyriform and unequal in shape with 4 to 6 filaments. Sporoplasm was finely granular and occupied most of the extracapsular cavity of spore.

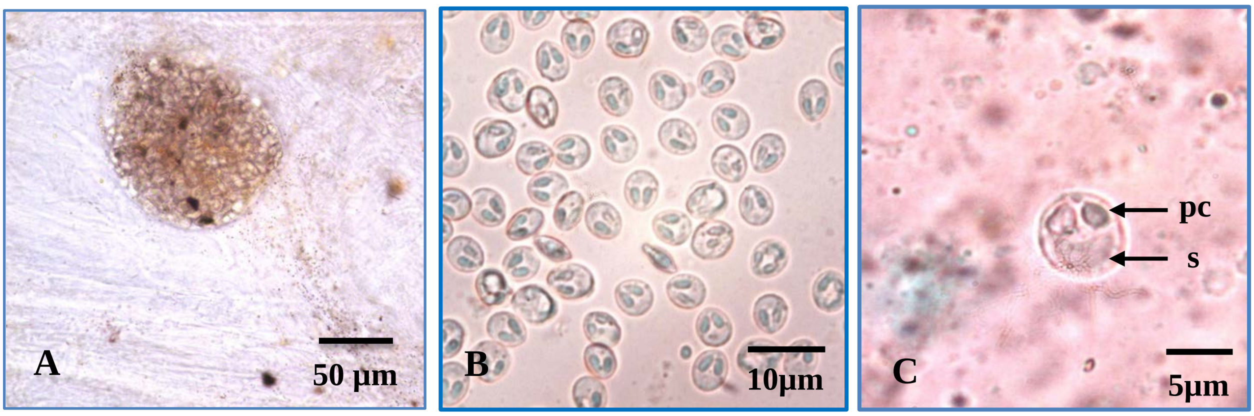

Plate 1 *Myxobolus rohitae* recorded from the muscle of *Cirrhinus mrigala* (Wet mount)
(A) Cyst of *M. rohitae* infested to the muscles of *C. mrigala*, (B) Spores of *M. rohitae* recorded in the muscles of *C. mrigala*, (C) Spore of *M. rohitae* (pc = polar capsule, s = sporoplasm)

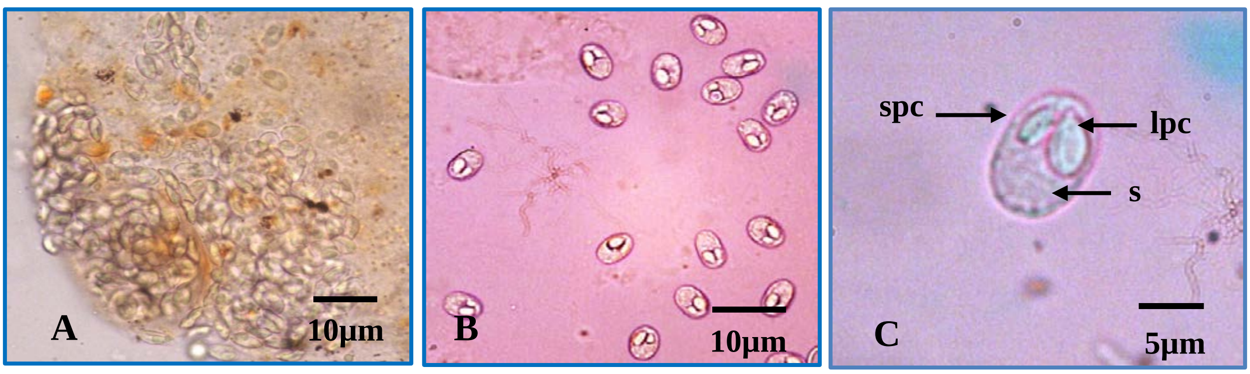

Plate 2 *Myxobolus* sp. recorded from the intestine of *Cirrhinus mrigala* (Wet mount)

(A) Plasmodia of *Myxobolus* sp. infested to intestine of *C. mrigala* (B) Spores of *Myxobolus* sp. (C) Spore of *Myxobolus* sp. (lpc = large polar capsule, spc = small polar capsule, s = sporoplasm)

Prevalence and mean intensity of *Myxobolus* spp.

The prevalence and mean intensity of *Myxobolus* species infested in *C. mrigala* were recorded from September 2018 to August 2019. The prevalence of *Myxobolus rohitae* in muscles was found 3% in December 2018 and gradually increased to the highest prevalence 48% in July 2019 and slightly decreased to 28% in August 2019. The prevalence of *Myxobolus* sp. was initially recorded only 8% in November 2018. The prevalence of *Myxobolus* sp. infection was 14% in March 2019 and it was markedly increased to 54% in May 2019. Then, it was slightly decreased to 48% in June and minimally increased to 54% in July and 62% in August 2019.

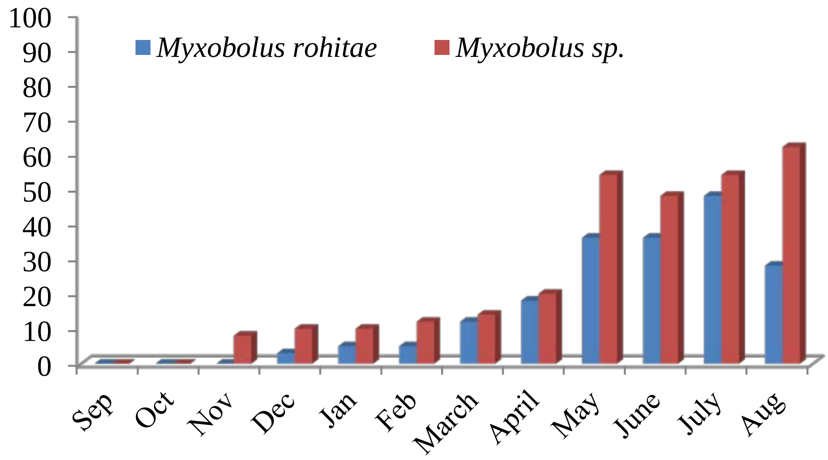

Figure 1 Prevalence of myxosporean infections in the muscles of *Cirrhinus mrigala*

The mean intensity of *Myxobolus rohitae* was recorded at stage 1 in December 2018 to March 2019 and gradually increased to highest intensity (2.5) in June 2019. *Myxobolus* sp. infested in muscle with mean intensity 1 in November 2018 to February 2019 and gradually increased to 1.5, 2.1, 2.9, 2.5, 2.6 and 2.6 in March, April, May, June, July and August 2019 respectively.

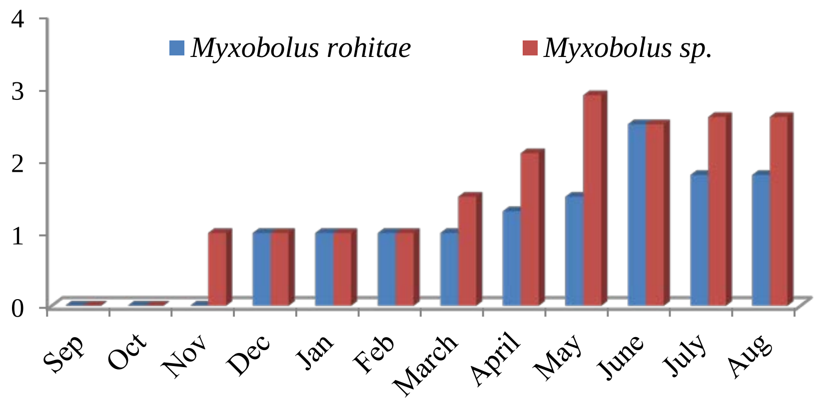

Figure 2 The mean intensity of myxosporean infections in the muscles of *Cirrhinus mrigala*

Histopathological Changes of the Muscles infested with *Myxobolus* species

In the study, plasmadia were found intracellularly in muscle cells of infested fish. In infested muscle, loss of epidermis, dermis split and separated from muscle, parasitic infestations and hemorrhage were found in muscle layer (Plate 3). Epidermis sloughed off from dermis, dermis split from muscle, myotomes were necrotic, *Myxobolus* spesies cyst and vacuums were found in many places (Plate 3, A).

In the same way, histopathological lesions and attached of *Myxobolus* spesies cyst were observed in fibrous connective tissue of dermis layer (Plate 3, B). Muscle tissue showed histoarchitectural loss in fishes that were characterized by the increased changes in the muscle fiber along with intercellular edema, necrosis, atrophic myocytes and mass of *Myxobolus* sp. mature spores (Plate 3, C). Whereas muscle structure was almost normal except the *Myxobolus* spesies cyst and any disintegrated muscle could not be seen obviously (Plate-3, D). Although, the muscle seems to be lost the myoseptum that separate within the myotomes in the muscle tissue (Plate 3, E). Each muscle bundle was irregular in shape and possesses peripheral nuclei and encysted parasites were enveloped by a loosened fibrous tissue (Plate 3, F). The damaged muscle caused by myositis which was characterized with a defect in the muscle. Necrosis is the death of cells caused by acute cell damage.

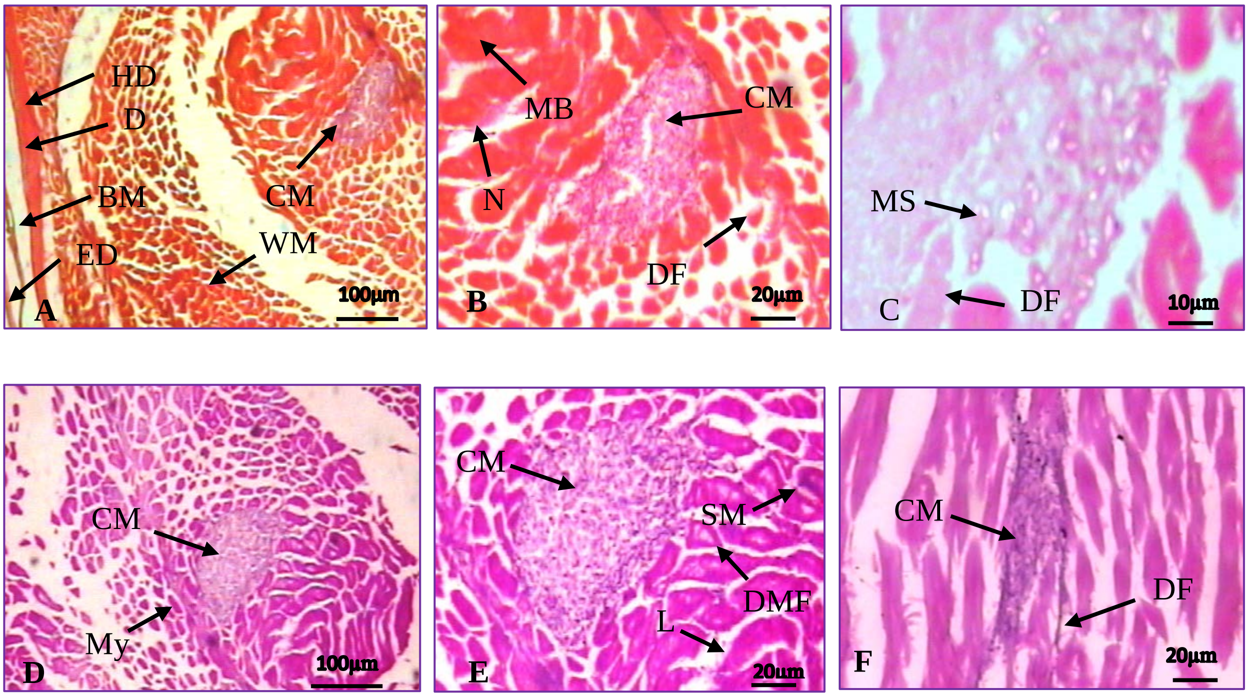

Plate 3 Histopathology in muscle tissues of *Cirrhinus mrigala* caused by *Myxobolus* species infestations (A) Developing intracellular plasmodium filled by sporogonic stages and young spores of *Myxobolus* species in a cross-sectioned muscle cell of *Cirrhinus mrigala.* (ED=Epidermis, D=Dermis, BM=Basement membrane, HD=Hypodermis, WM=White muscle, CM= cyst of *Myxobolus* sp.) (B) A mass of *Myxobolus* sp. spores in the intermuscular space, released from a disrupted plasmodium of the damaged host cell. (N=Necrosis, MB=Muscle bundle, CM= cyst of *Myxobolus* sp., DF=Disintegrated fibers) (C) Mass of *Myxobolus* sp. mature spores and disintegrated muscle (MS=Mature spore of *Myxobolus* sp, DF=Disintegrated fibers) (D) A *Myxobolus* species plasmodium located in the intermuscular septa of the muscle of *Cirrhinus mrigala*. (My=Myofbrils, CM= cyst of *Myxobolus* sp.) (E) *Myxobolus* sp. spores among cross-sectioned muscle cells of roach *Cirrhinus mrigala* encapsulated by a thick connective tissue layer (MC= *Myxobolus* sp. cyst, L=Lesion, DMF=Disintegrated myofibrils, SM=Split muscle) (F) Longitudinal section of the muscle cells of *Cirrhinus mrigala* infected with elongated mature plasmodia of *Myxobolus* sp. (MC= *Myxobolus* sp. cyst, DF=Disintegrated fibers)

Discussion

To establish the identity of the present specimens, they were compared with all the species of *Myxobolus* described so far, and therefore including all the species described from hosts in Myanmar. Lom and Dykova, 2006 estimated that, the world of myxosporidia fauna was composed of about 2180 species gathered within 62 genera among which the genus *Myxoblus* represented about 36.33% of species (792 species). *Myxobolus* is the predominant species group within the phylum Cnidarian. Most of the species infect primarily fish, both freshwater and marine species and a few numbers of species were found in amphibians (Eiras *et al*., 2005). The importance of site selection as a diagnostic characteristic for myxosporean species infecting gills, fins and kidneys have demonstrated (Molnar, 2007). The relatively low number of reported muscle infections might be due to the technical difficulties of examining muscles. Examining the myxosporeans infection in the muscle is rather difficult. While even small plasmodia can readily be studied in the gills, plasmodia infecting the muscles must be detected in muscle samples. In Asia, *Myxobolus lentisuturalis,* a pathogenic species infecting the gibel carp in China, is the best-studied species developing intracellularly in muscle (Dykova *et al*., 2002). In Indian, 7 out of the 97 *Myxobolus* species have been located in muscle (Kalavati and Nandi, 2007). The present study represents the first report of *Myxobolus* infection in the muscle tissues of *Cirrhinus mrigala* in Myanmar.

Passage of myxozoan parasites into human feces has been described (Reis *et al*., 2019). They reported that 13% of the 97 samples, the parasitological examination of fecal samples from adults (22 to 71 years old) resulted in the detection of myxozoan spores in the Amazonas State, Brazil. In this state, consumption of fish is very high, and is the main source of protein, particularly for people living in rural areas and in riverine communities (Lopes *et al*., 2016). Another Myxozospoean, *Kudoa septempunctata*, was responsible for outbreaks of food poisoning caused by the consumption of raw fish in Japan (Kawai *et al*. 2012). Consuming raw, undercooked, or smoked fish causes dipyllobothriasis in humans due to *Diphyllobothrium latum*, a cestode (Emmel *et al*. 2006). The habit of eating raw fish such as sushi and sashimi has spread throughout the world. Fish consumption has increased worldwide including Myanmar because it is a healthy source of nutrients that is rich in proteins, minerals, and essential fatty acids. Therefore, it is possible that food-borne illness caused by myxosporean parasites will occur in Myanmar.

In the present study, the plasmodia of *Myxobolus* sp. containing with mature spores in the central part of the plasmodia were observed in the muscle tissues of *Cirrhinus mrigala*. Myxosporean spores have been shown to be very resistant to a range of environmental conditions and can survive entrance through the alimentary tracts of piscivorous vertebrates (El-Matbouli *et al*., 1991). They observed that myxosporean cysts and spores have been damaged by freezing or cooking due to the release of proteolytic enzymes by postmortem myoliquefaction of fish fillets.

In the present study, parasitic prevalence of *Myxobolus rohitae* in muscles was highest (48%) stated as “stage 2” in July 2019. *Myxobolus* sp. was found in the muscles with the highest prevalence 62% stated as “stage 3” in August 2019, which was nearly similar to the finding of Deva (2016). The author stated that *Myxobolus* sp. infection in the gills of *Labeo rohita* reached peak stage (52%) during the raining season.

The shape and dimension of *Myxobolus* spp., in the present study were compared to those of other *Myxobolus* spp. reported by Eiras *et al*. (2014). *Myxobolus rohitae* recorded in the present study is superficially similar to *Myxobolus eirasi* infected in caudal fin and *Myxobolus guangzhouensis* infested in scales of *Cirrhinus mrigala*. The shape and size of *Myxobolus* sp. detected in this study is also similar to *Myxobolus* sp. A total of 7 fish infected in gills and kidneys of *Cirrhinus mrigala* from Kantawgyi Lake were recorded by Pa Pa Win (2007) and

Myxbobolus sp. infected to the gills of *Labeo rohita* from Lay Daung Kan Fish Farm was recorded by Su Su Mon (2014). Although length and width of the spores of *Myxobolus* species were not differed from those species; the size of polar capsules was slightly different.

The histological changes associated with the present infection caused slight distention of the muscularis and led to replacement of the muscle tissues with developing plasmodia. In the infected portions of the muscles, histological observation showed that some alterations of muscle tissues like necrosis and abnormalities in the muscle fiber. Hence, the severe infection may indicate a health risk to the infected fish. The invading ectoparasites cause significant necrotic changes in skin and muscle tissue, produce lesion and ultimately result in the formation of dermal ulcers (Ahmed, *et al*., 2007). Furthermore, Das and Chandra, (2018) observed that partial or total loss of epidermis and dermis, dermal splitting, necrosis, pyknosis, vacuolation, hemorrhage and presence of parasites and fungal granuloma occurred mainly in skin and muscle pathology. In addition, almost similar pathological symptoms in skin and muscle of various freshwater species in Bangladesh were also found by Hossain, *et al*. (2009) and Moniruzzaman (2000). The result agreed with the finding of Chandra *et al*. (2012) who observed that pathologically, skin and muscle were almost normal structure in carps.

According to Golder, *et al*. (1987) presence of *Chilodonella* sp., *Trichodina* sp., *Dactylogyrus* sp., *Ichthyophthirius multifiliis*, myxosporean spores and fungal granuloma with necrosis, pyknosis and hemorrhage in muscle of *Nandus nandus* were significant pathology as recorded from ponds. *Myxoblus lintoni* caused marked changes in the epidermis and hypodermis of the skin of the host *Cyprinodon vreiegates*, characterized by invasion of fibroblasts (Dykova and Lom, 1978). According to Molnar and Kovacs-Gayer, (1985), *Myxobolus* sp. is a typical intracellular parasite of muscle cells, which their spores are found in other organs. The spores will spread to the organs throughout blood circulation.

In the present study, histopathological lesions and attached *Myxobolus* sp. cyst were observed in fibrous connective tissue of dermis layer. Each muscle bundle is irregular in shape and possesses peripheral nuclei and encysted parasites are enveloped by a loosened fibrous tissue. Furthermore, disintegrated tissue like intercellular edema, necrosis, atrophic myocytes and mass of *Myxobolus* sp. spores in the muscle tissue of host fish were found out. Maftuch, *et al*. (2018) said that necrosis is the death of cells caused by acute cell damage. It was characterized by the death of muscle tissue that still attached to the fish body surface. Similarly, Plumb, (1994) said that necrosis was characterized by the death of cells or tissue that accompanied in cell degeneration in animal life and it was the final stage of irreversible degeneration. Therefore, clinical signs and pathogenesis in muscle and other important organs of host fish might be due to the *Myxobolus* infestations.

The histopathological findings of the present study were consistent with the infection of the muscles of *Cirrhinus mrigala* by *Myxobolus* species, with clear evidence of clinical signs in the fish specimens. The necropsy revealed extensive damage to the host organism, with fibrocystic infections established in the muscle fibers, resulting in histopathological finding.

Conclusion

The muscles of *Cirrhinus mrigala* collected from Yezin Fishery Station were infected with *Myxobolus* species. High prevalence of infection was recorded during the raining season. Histopathological lesions and attached *Myxobolus* species cyst were observed in fibrous connective tissue of dermis layer. The loss of epidermis, dermis split and separated from muscle, and hemorrhage were found in muscle layer of *Cirrhinus mrigala*. These parasites were infecting economically important fish species although not seriously dangerous for humans. Thus, an important action to prevent the spread of these parasites is removing dead and infected fish, and using water from parasite-free sources.

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