

Histopathological observations on *Thelohanellosis* in Indian major carp

Dr. Pratibha Pathak

P.G.Department of Zoology, LNMU., Darbhanga, Bihar, India.

Abstract:

The current study examines the parasitic effects of *Thelohanellus* sp. a myxosporean parasites on Indian major carps. Only symptomatic and moribund samples of diseased fishes comprised of fry/fingerlings and adults of IMC were collected and were brought to the laboratory for patho-morphological and patho-anatomical examinations. Photomicrographs of the most characteristic regions of histopathological lesions in the stained tissues of diseased fish samples were taken. Examination of cysts in smear preparations confirmed the nature of the parasitic spores which were oblong and spindle shaped. Gross pathology revealed body emaciation and anaemia. Such cyst formation in the skin induced severe histopathological lesions in the dermal layer the damage caused by the parasites in the skin of the affected fishes was sufficient enough to cause mortality in them because of the restriction of the respiratory surface areas. The myxozoan parasite was observed predominantly in the nursery and rearing ponds. Fry, fingerlings, juveniles and adults of Catla and mrigal were found highly susceptible to this disease than rohu.

Key: Catla, histopathology, *Thelohanellus*, *Myxobolus*.

INTRODUCTION:

In most of the fish farms, it was observed that among all fish pathogens, parasitic infestation has been the major cause of concern because of high morbidity and slow growth rate. These parasites were difficult to be removed from the culture system, causing significant setback to freshwater aquaculture. Under poor water quality conditions or stressful environment, these parasites multiply rapidly there by affecting fishes with high morbidity (Mishra SS, *et al.* 2017).

Achmerov (1955) described several species of myxosporean parasites including *Thelohanellus nikolskii* isolated from cysts presented on the fins. The same parasite was described by Hoshina and Hosoda (1957) in carp fingerlings in fish ponds in Japan and named it *Thelohanellus cyprini*. Ivasik and Karpenko (1967) reported that *Thelohanellus nikolskii* entered from the western parts of Ukraine to belarus by means of wild carp, The same authors reported that wild carp and their hybrids transmitted the parasite via the river amur to the Trans Siberian area (in 1963). *Thelohanellus nikolskii* infection in europe was first reported in Hungary by Janey (1979) as cysts found on the fins of carp fingerlings. The parasite was introduced from the far East and causes each year an intensive infection of carp fry in Hungary (Molnar and Kovacs-gayer, 1981-1982; Molnar, 2002). In Serbia *Thelohanellus nikolskii* was reported in the beginning of the eighties (Ćirković *et al.*, 1983) and is still present. In moldova, T. nikolskii infection was first recorded by Trombitsky *et al.* (1983, 1990). The first cases of thelohanellosis in poland was described by Antychowicz (2003) and Kramer and Przybysz (2004). In Czech Republic, Novotny and Smolova (2006) was first who reported *Thelohanellus nikolskii* infection of carp fingerlings which were imported in this country.

It is essential therefore, to obtain timely and precise diagnosis of any fish disease outbreak in culture fishery operations. Throughout visual examination for external signs of the disease at the pond site should be followed by detailed Pathomorphological, Pathoanatomical and microscopical, examinations of squas and permanent stained preparations of the tissue samples of diseased fishes in the laboratory (Ramudu KR, Dash G, Abraham T., 2013). Disease diagnosis also require analysis of water and soil sample from the ponds from where diseased fish samples are collected for diagnostic purposes.

The current study examines the parasitic effects of *Thelohanellus* sp. a myxosporean parasites on Indian major carps. It was observed predominantly in the nursery and rearing ponds. Fry, fingerlings, juveniles and adults of rohu, catla and mrigal were found susceptible to this disease. The infection may visualize with necked eyes and need to manage promptly to secure the health of Indian major carps.

MATERIALS AND METHODS:

The samples were collected from hatcheries and ponds of Madhubani and Darbhanga, and brought to the laboratory for patho-morphological and anatomical examinations. Collected samples were comprised of identifying and locating any visible external lesions formations of cysts on skin of major carps.

Fixed tissue samples were then processed and paraffin embedded blocks of all the tissues was prepared using the standard histological methods (Luna, 1968). blocks were cut into serial sections (5-7 μ thick) by a rotary microtome. Mallory Heidenhain (Cason) stain used.

RESULTS:

Fish species affected

Fry and fingerlings (100-150 mm size) of *Catla catla* (Ham.), *Cirrhinus mrigala* (Ham.) and *Labeo rohita* (Ham.) were found affected by this disease during seed rearing periods. Catla and mrigal were found highly susceptible to this disease than rohu. Mass mortalities of catla and mrigal seed were encountered in few occasions due to this disease during the period of observation.



Figure:1. White round compact cysts found on various parts of the body of *Cirrhinus mrigala*.

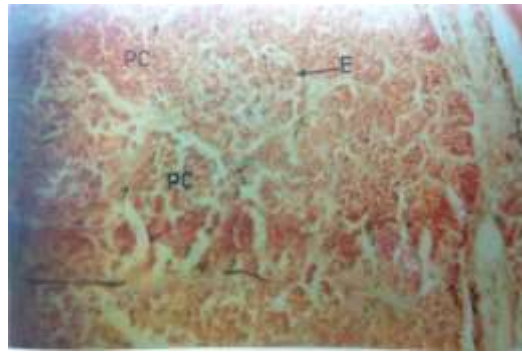


Figure:2. Skin of *C. mirigala* infected with *Thelohanellus sp.* showing the epidermis has numbers of parasitic cysts(PC), epitheloid (E). H.E. stained. X 200

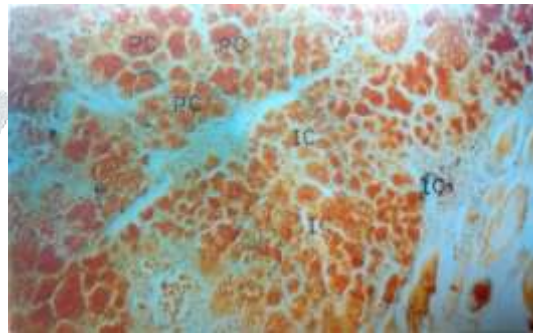


Figure:3. Skin of *C. mirigala* infected with *Thelohanellus sp.* showing dermis and subdermal areas of the skin section has parasitic cysts (PC),inflammatory cells (IC) around the cysts. Cason stain X200

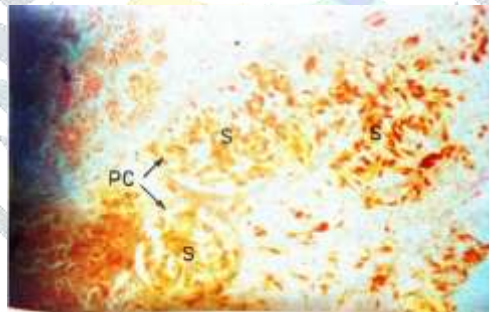


Figure:4. Skin of *C. mirigala* infected with *Thelohanellus sp.* showing parasitic spores and parasitic cyst (PC).Cashion stain, X 200

Clinical symptoms and Gross pathology

The affected fishes had white round compact cysts on various parts of the body excepting the gills (Fig. 1). The cysts (about 1-2 mm diameter) were seen protruded from the body wall in many samples. The affected fishes had excessive mucus or slime over the body. Examination of cysts in smear preparations confirmed the nature of the parasitic spores which were oblong and spindle shaped. Gross pathology revealed body emaciation and anaemia. Internal organs like kidney, liver, spleen and intestine did not show any altered appearance and presence of spores in the smear preparations.

Histopathology

In tissue section the regions of cyst formation showed significant pathological changes and cellular reactions. Epidermis was found penetrated by parasite and large numbers of parasitic cysts containing the spores inside when peel off were found in the subdermal connective tissue which was virtually packed with parasitic cysts of various sizes. The connective tissue forming the cyst-wall was so changed that it looked like epitheloid in nature in many places (Fig. 2, H.E. stained). The entire dermis and subdermal areas of the skin section was full of parasitic cysts (Fig. 3, Cason stain) causing degenerative changes and there were infiltration of inflammatory cells around these cysts. Cason stained section of the skin (Fig. 4) confirmatively demonstrated the parasitic spores. In some zones of the histological section of the skin, the spores of *Thelohanellus sp.* found outside the cyst-wall which was seen in broken or decomposed condition liberating the spores in the neighborhood. The dermis had also multifocal oedematous areas and some blood vessels were seen congested. Although the muscular layer in the skin did not show any parasitic cysts, but the muscle bundles were oedematous and in many places the muscle fibres exhibited degenerative and necrotic changes. Histological examinations of the cellular status of other organs like kidney, liver, gills and intestine of the same samples from which skin sections showed parasitic cysts, did not reveal either the presence of the parasites in them or any tissue level changes. However, the damage caused by the parasites in the skin of the affected fishes was sufficient enough to cause mortality in them because of the restriction of the respiratory surface areas.

Diagnostic histopathological lesion identified

Formation of parasitic cysts containing spores in the dermal and subdermal connective tissues of the affected skin areas were the histologically recognizable pathological lesions at the tissue level in the present case of the disease reported upon. This disease was found to be caused by the myxozoan parasite, *Thelohanellus sp.*

DISCUSSION:

Thelohanellus sp., the myxozoan parasite causing this disease was found to damage the skin of the affected fishes by formation of cysts of various sizes on many parts of the body. Such cyst formation in the skin induced severe histopathological lesions in the dermal layer and consequently damaged the cutaneous respiratory process of the affected fishes. Fry and fingerlings of the Indian major carps perform substantial respiration through their skin. So, the parasitic cysts adversely affected the respiration process and caused mortality in acute stage of infestation. Histopathological the whole sub-dermal layer was full of the pansporoblasts of the parasites which were located in the connective tissue.

The whole connective tissue at the places of cyst formations became so changed that it looked epitheloid in nature surrounding the cysts. Such subdermal cysts of *Thelohanellus sp.* In the connective tissue of the skin of cyprinids and white fish in the Soviet Union were also recorded by Shulman & Shtein (1962) thus corroborating the findings of the present studies. The first cases of thelohanellosis in Poland was described by Antychowicz (2003) and Kramer and Przybysz (2004). In Czech Republic, Novotny and Smolova (2006) was first who reported *Telohanellus nikolskii* infection of carp fingerlings which were imported in this country. Moshu and

Molnar (1997) described for the first time *Thelohanellus* infection of scales in 2-4 years old European wild carp (*Cyprinus carpio carpio*) collected from water reservoirs of Moldova. Ćirković *et al.* (2009), Milošević (2009) and Novakov (2013) have detected and described *Thelohanellus nikolskii* infection of the scales in common carp (*Cyprinus carpio*) from Serbian fish farms. Changes are visible in the form of cysts on the scales reaching up to 3 mm in diameter. By analyzing the pathohistological structure of the plasmodia from the fins and scales an almost identical structure, as described by Moshu and Molnar (1997) can be seen. By molecular characterisation of the parasite from the scales was confirmed that the etiological cause was *Thelohanellus nikolskii* Achmerov, 1955 is the same for both forms of the disease which occurs on the scales and on the fins (Novakov, 2013).

Thelohanellus sp. has been reported to form parasitic cysts in other fish tissue also. Van Duijn (1972) mentioned cyst formations in the gills, kidney and spleen of Tench (*Tinca tinca*) and in the muscles of Bitterlings (*Rhedous amarus*). Kalavati *et al.* (1981) reported *Thelohanellus sp.* from the gills of Indian major carps catla, rohu and mrigal but did not mention about the histopathological lesions caused by the parasite. In the present observation the parasite was found only in the skin, gill and other internal tissue like kidney and spleen did not show the parasitic presence indicating that the target tissue if this parasite in the fry and fingerlings of the Indian major carps was the skin. Molnar (1982) studied the hostopathological changes in the common carp in Hungary due to *Thelohanellus nikolski* and observed that the parasite produced larger cysts on the fins of the fry which were surrounded by a thick capsule of connective tissue strengthened by a cartilaginous elements. Dykova and Lom (1987) while carrying out observations on the host cell hypertrophy induced by contact with trophozoites of *Thelohanellus pyriformes* found that the trophozoites in the form of polysporic plasmodia developed in the gill filament arteries. The plasmodia were found coated with endothelial cells of the host (Molnar, 2002). One of the cells was enclosed within the plasmodium and became greatly hypertrophic without being pervaded by the parasites. The plasmodium development of *Myxobolus diversus* is associated with connective tissue cells that are also capable of forming chondrifying collagenous extracellular product (Molnar, 2003).

In the present study the host reactions due to the parasite concentration and its cyst formations in the skin were significantly noticeable because of excessive infiltration of mononuclear cells particularly macrophage and lymphocytes around the pockets of parasite cysts. These infiltrating cells were probably pressed into service by the host fish as its defense against the pathogen. The matured cysts in many places of the subdermal layer of the skin were found broken and the spores inside were seen scattered in the connective tissue. Such spores in the very environment from of infestation to other fish in the population.

Although muscle layers of the skin in the affected fishes did not show any parasitic cysts, but the muscle bundles showed oedematous and degenerative changes in and around the sites of cysts formations. This was probably due to the pressure atrophy which the parasites cysts caused and the toxins released by the parasites when the cysts broken and got released into the surroundings. Another important histopathological lesion this

parasite caused in the host fish is in some of the blood vessels of the dermis which became dilated and congested very much. In some blood vessels the endothelial lining was found necrotic and the lumen of the vessel was filled with red blood cells, some infiltrating cells and eosinophilic fibrous materials. This indicated the inflammatory reactions which the parasite caused in the host fish during the course of the pathogenesis (Rogers & Gaines, 1975).

The disease was found to cause large scale mortalities of fry and fingerlings of catla and mrigal especially at the time of seed rearing operations during the period of observations. Rohu was found to be very mildly affected by this disease. Incidences of *Myxozoan* parasitic diseases including thelohanellosis were earlier reported to cause mortalities and economic damage to fish farmers and aquaculturists by Chakravarty, 1943; Basu, 1950; Tripathi, 1952, Chaudhuri and Chakravarty, 1970, Seenappa and Devraj, 1990, Rao *et al.* 1990, Kumar and Dey, 1991 and Rao, 1991. But those studies did not throw any light on the way the disease caused damage to the host fish histopathological and the pathogenesis and localization of the parasites in the vital tissue of the fish. The present observations on this disease clearly indicated and demonstrated the involvement of the pathogen and its pathogenic effects to the host fish pinpointing definite tissue (skin) which the parasite invaded and the tissue level lesion caused therein. This is definitely an addition to the knowledge on this important fish disease afflicting our cultivable carps in culture operations and the study has given additional information for strengthening the diagnosis of this disease which is very vital and important for successful management of the disease in farm ponds.

This disease incidence was recorded mostly from ponds tanks where organic load in the bottom was sufficiently higher. *Myxozoan* parasites are known to remain in pond bottom for a considerably long time and thrive well in presence of high organic load. The parasites find ideal conditions for development and propagation when the ponds are stocked with high densities. High stocking densities and malnutrition cause stress to the stocked population and magnify the chance of disease outbreaks sometimes assuming epizootic proportions.

Regarding control of this disease, no therapeutic drugs or chemicals have so far been found effective against the parasites causing the disease. However, prophylactic and preventive measures in the form of applications of bleaching powder at the rate of 50-60 ppm in the pond before stocking have culture ponds (Kumar & Dey, 1991). Schaperclaus (1986) suggested that when this parasitic infestation occurs naturally in ponds the affected fishes should be caught and destroyed. Even those fish which survive after disease outbreak can harbor millions of the parasites. After the fish die, the spores become liberated in the environment and then can cause infestation to new batch of fishes thus continuing the disease process.

CONCLUSION:

The present observations on this disease clearly indicated and demonstrated the *Thelohanellus sp.*, the myxozoan parasite causing this disease was found to damage the skin of the affected fishes by formation of cysts of various sizes on many parts of the body. Such cyst formation in the skin induced severe histopathological lesions in the dermal layer and consequently damaged the cutaneous respiratory process of the affected fishes.

The findings accomplished that disease, thalohanellosis caused by *Thelohanellus sp.*, the myxozoan parasite was observed predominantly in the nursery and rearing ponds. Fry, fingerlings, juveniles and adults of Catla and mrigal were found highly susceptible to this disease than rohu.

ACKNOWLEDGEMENT:

The authors are thankful to the Department of Zoology, LNM University, Darbhanga, for the provision of laboratory facilities used in this study.

REFERENCES:

Achmerov AC, 1955: Ways of the origin of myxosporidia species of the genus *Thelohanellus* Kudo from amur wild carp, Dokl Akad Nauk SSSR, 105, 1129-32 (in Russian).

Antychowicz J., 2003: Carp diseases in poland caused by myxosporea parasite (buetschli 1881), Medycyna Wet, 59, 762–6.

Chakravarty, M. M., 1943: Studies on myxosporidia from common food fishes of Bengal. Proc. Indian. Acad. Sci. (B), 18 : 21 -35.

Chaudhuri, S. R. and M. M. Chakarvarty, 1970 : Studies on the food fishes of Bengal. 1. three new species from ophicephalus punctatus (Bloch) Acta. protozool. 8 : 167 - 173.

Ćirković M, Milošević N, Jovanović M, Jeremić S, Radosavljević V, 2009 : *Thelohanellosis* of the scales in the two-year-old cultured common carp (*Cyprinus carpio*), 14th eafp International Conference, prague, book of abstracts, 251.

Ćirković M, Petrović Z, Jovanović B. 1983: *Thelohanellus* of carp in the territory of Yugoslavia. - Ist International symposium of ichthyoparasitology parasites and parasitic diseases of fish, Č. budejovice, book of abstracts, 38. 7.

Dykova, I. and J. Lom, 1987: Host cell hypertrophy induced by contact with trophozoites of *Thelohanellus pyriformis* (Myxozo : Myxosporea) . Arch . Protistenkd 133 (3/4) : 285 -293 .

Ivasik M, Karpenko M, 1967: The cange of parasite fauna of *Cyprinus carpio haemotopterus* Tem. Rt. Schl. At. Its. Introduction in fish ponds of the Ukraine (in Russian), Parazitologija, 1, 149-50. 15.

Janey G, 1979: The occurrence of *Thelohanellus dogieli* achmerov 1955 (Myxosporidia) on carp (*Cyprinus carpio*) in fish ponds in Hungary, Parasitol Hung, 12, 19-2.

Kalavati, C., B. V. Sandeep and C. C. Narasimhamuri, 1981: Two new species of myxopodians, *Myxosoma channai* n.sp. and *Myxobolus tripathii*, n.sp. from freshwater fishes of Andhar Pradesh. *proc . India Acad Sci.* 90 (1): 61 -78.

Kramer I, Przybysz M, 2004: Myxobolosis and Thelohanellosis – fish diseases caused by Myxosporea, *Przegląd Rybacki*, 1, 22–7.

Kumar, D. and R. K. Dey, 1991: Fish diseases in India, *Aquaculture Productivity*. Ed. V. R. P. Sinha H. C. Srivastava, Oxford & IBH publishing Co ., Pvt. Ltd . pp . 315 – 343.

Luna, G. 1968 : *Manual of histologic staining methods of the Amerd Forces Institute of Pathology*, 3rd Edition, McGraw–Hill Book Company, New York .

Mishra SS, Das R, Choudhary P, Debbarma J, Sahoo SN, et al. 2017: Prevalence of Fish and Shrimp Diseases and Use of Various Drugs and Chemicals in Indian Aquaculture for Disease Management. *J Fish Aqua Dev: JFAD-129*. DOI: 10.29011/JFAD-129. 100029

Molnar K, 2002 : Differences between the european carp (*Cyprinus carpio carpio*) and the colored carp (*Cyprinus carpio haematopterus*) in susceptibility to *Thelohanellus nikolskii* (Myxosporea) infection, *Acta Vet Hung* , 50, 51-7.

Molnar, K. 1982 : Biology and histopathology of *Thelohanellus nikolskii* (Myxosporea : Myxozoa), a protozoan parasite of the common carp (*Cyprinus carpio*). *Z. Parasitenkd.* 68 (3) : 269 -277.

Molnar K, Kovacs-Gayer, 1981-1982 : occurrence of two species of *Thelohanellus* (myxosporea: Myxozoa) of Far-East origin in common carp populations of the Hungarian fish farms, *Parasit Hung*, 14, 51-5.

Molnar, K., S. Csaba, 2003 : Infection in the goldfish *Crassius auratus* caused by *Myxobolus diversus* (Myxospora). *Folia parasitologica*, 50:29-34.

Novakov N., 2013 : Fish diseases caused by species *Thelohanellus nikolskii*, University of Novi Sad, Doctoral thesis, (in Serbian).

Novotny L., Smolova J, 2006 : *Thelohanellus nikolskii* in imported fingerlings of common carp (*Cyprinus carpio*) in the Czech Republic - a case report, *Bull Eur Ass Fish Pathol*, 26, 3, 128.

Ramudu KR, Dash G, Abraham T., 2013: Parasitic study of *Cirrhinus mrigala* (Hamilton, 1822) in Selected Districts of West Bengal, India. *International Journal of Advanced Biotechnology and Research*; 4:(4):419-436.

Rogers, W. A. and J. L. Jr. Gaines : 1975. Lesions of protozoan diseases of fish . In : *The pathology of fishes* (Ed. by W. E. Ribelin & G. Migaki). The University of Wisconsin Press. Madison , pp.117 -141.

Rao, M. B. 1991 : Ways of tackling problems of fish and shrimp diseases in culture sector. Fishing Chimes. 11 (3) : 8 -13.

Rao, K. G., R. Ramkrishna and V. Rao, 1990 : Observations on the prevalence of fish diseases in commercial farm ponds of Andhra Pradesh. Abstract. proc. 2nd Indian Fisheries Forum May, 27 -31, 1990. Mangalore, Karnataka, India, pp. 118.

Schaperclaus, W., 1986 : Fish diseases. Volume 2, English Translation by M. S. R. chari & V. S. Kothekar, Oxonium Press Pvt. Ltd. New Delhi, 1991.

Seenappa, D. and K. V. Devraj, 1990 : parasitic infestations on younger stages of cultivable carps in fish seed farms. Abstract. proc. 2nd Indian Fisheries Forum. May, 27 -1, 1990, mangalore, Karnataka, India. pp. 121.

Shulman, S. S. and G. A. Shtein, 1962. Protozoa. In : Key to parasites of freshwater fish of the USSR (Translated from Russian by the Israel program for Scientific Translations. Jerusalem, 1964). pp. 5-236.

Tripathi, Y. R. 1952 : Studies on Indian Fishes. Protozoa Myxosporidia together with a checklist of parasitic protozoa described from India fishes. Rec. Indian Mus. 50(1) : 63-68.

Van, Duijin. 1972 : Diseases of fish. Butterworth & Co. (publishers) Ltd., London.

