VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 1174-1178 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Characterization of Microsporidian Infection in *Labeo rohita* by Using Scanning Microscopy

Jaiswal Kamal , Mishra Suman and Meena Vikram Singh*

Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

**Corresponding Author*

Received: 2nd October, 2023; **Accepted:** 22nd November, 2023; **Published online:** 28th November, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.130

Abstract: The present study aimed to investigate the role of Microsporidian infection in *Labeo rohita*, a well-known species of fish of the carp family especially found in rivers in South Asia. Fish were collected from different regions of Lucknow and further investigated for presence of microsporidian infection by using standard protocol. Scanning electron microscopy (SEM) revealed the spore as oval and cylindrical with 5.60 to 8.28 µm size. Changing climatic conditions acting upon the microsporidian sp. also affect spore size. Scanning electron microscopy revealed the ultra-structural characteristics of the microsporidia, however, the species identification is only possible with the help of molecular techniques. Data of our current findings propose that microsporidian parasite has a crucial role in the pathogenesis of host. Thus, the results of the present research open up new approaches for better understanding the role of microsporidia in fish infection. Further studies are warranted to elucidate the specific findings related to species and identify mechanism of parasitic induced infection.

Keywords: Microsporidia, *Labeo rohita*, Light microscopy, Scanning microscopy

Citation: Jaiswal Kamal , Mishra Suman and Meena Vikram Singh: Characterization of microsporidian infection in *Labeo rohita* by using scanning microscopy. Intern. J. Zool. Invest. 9(2): 1174-1178, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.130

Introduction

Food security is an emerging concern for every nation. India being the second most populous nation needs to ensure food security, for which diversification of food resources is crucial for sustainable livelihood. However, there has been constant decline in fish population. One of the significant factors that influence fish diversity, includes endoparasite like microsporidia that causes serious threats to fishes and cause immense harm to overall aquaculture industry. Microsporidian parasites are diversely distributed opportunistic obligate, eukaryotic and intracellular group of pathogens which infect different host ranging from invertebrates to vertebrates. The infection caused by them initiates unknown pathways and the mechanisms and have been the focus of research for some time, is in nascent stage.

Fish have an important role in maintaining life on earth as they are major contributor in ensuring food security. They have a key role in the maintaining aquatic biodiversity and is considered to be the necessary part of the aquatic ecosystem. Much attention has been given on managing populations of fish health. However, nowadays, many fish population are decreasing day by day due to parasitic invasions (Mosquera *et al.*, 2003). Microsporidia are small, single celled, spore-forming microorganisms (extremely evolved fungus) and an obligate intracellular parasite of eukaryotes which chiefly parasitize on insects (e.g. silkworms, bees and mammals) (Keeling, 2009). The presence of polar filaments in spores, places the parasite under the phylum Microsporidia (Boucias and Pendland, 1998).

Microsporidia are known to infect fresh, marine, and brackish water fish in their natural environment, as well as in experimental models, resulting significant economic loss due to liquification of muscles, growth retardation, unhealthy flesh (Fig. 1) rendering it unfit for human consumption (Grabda,1978; Lom and Nilsen, 2003; Joh *et al.*, 2007; Sanders *et al.*, 2012). Microsporidian infection has been detected in different organs of fish like muscles (McGourty *et al.*, 2007), gills (Kummari *et al.*, 2018), kidney (Didier *et al.*, 2004), liver and ovary (Matos *et al.*, 2003), peritoneal cavity (Casal *et al.*, 2008; Morsy *et al.*, 2013).

Microsporidia, being an intracellular parasite, contains a unique polar filament that invades the host cell. After invasion, microsporidian parasite multiplies inside the host cell through various developmental stages like merogony, sporogony, and sporoblast resulting in hypertrophy and bursting of the host cell (Sprague *et al*., 1992). Host cell induced spores infects new host and cells through vertical and horizontal modes of transmission (Lom *et al.*, 1995; Kent and Stewart *et al.*, 2003). Due to their intracellular mode of parasitic character, microsporidian parasites are highly specialized, as they contain genes that are necessary for metabolic pathways such as glycolysis, trehalose metabolism, and pentose-phosphate pathway. Genes for tricarboxylic acid and oxidative pathways are absent in microsporidian parasite. Mechanism of Glycolysis might provide ATP molecules and other essential metabolites that are necessary for its diverse metabolic activities to conduct different physiological processes of this organism (Keeling *et al.*, 2010).

Labeo rohita (rohu) being Indo-Gangetic riverine species is the natural inhabitant of northern and central India, and the rivers of Pakistan, Bangladesh and Myanmar. The compatibility of rohu with other major carps like catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) has made it an ideal species for polyculture systems. High growth potential, high food conversion ratio and high consumer preference have established rohu as the most important freshwater species cultivated in India, Bangladesh etc.

Labeo rohita (Hamilton) is the natural diurnal inhabitant found in ponds and rivers are bottom feeders, omnivorous fish, cannot be cultured in confined water and can tolerate wide range of temperature. *Labeo rohita* is a bottom feeder and due to having arranged slightly downward mouth with thick lips, they feed on phytoplanktons, weeds, aquatic plants etc. The production from culture is badly affected by infection of parasites. The parasites multiply under suitable condition of temperature, host physiology and water quality of the river or pond. In various findings, microsporidian infection has been reported in *Labeo rohita* (Dey and Kumar, 1986). The population of this fish is steadily declining due to over exploitation, parasitic infestation, loss of habitat, introduction of foreign species, disease, pollution, siltation, poisoning, dynamite, and destructive methods of fishing from the last 10 years.

Present study was conducted to perform the morphological identification of microsporidia isolated from *Labeo rohita* using scanning electron microscopy. However, studies related to the

behavior of infected fishes need more explorations and evidences.

Materials and Methods

Random collection of *Labeo rohita* from different regions of Lucknow, India was done during 2021 July to March 2022.

Identification and Detection of Microsporidia:

Labeo rohita were brought to the parasitology laboratory of Babasaheb Bhimrao Ambedkar University and were kept at -20 C. After 40 min, midgut of *Labeo rohita* was homogenized using potassium carbonate solution and temporary slide was prepared to visualize the presence of spores. After confirmation of the infected *Labeo rohita*, the homogenised solution was centrifuged by following the protocol mentioned by Tsai and Wang (2001) to get the crude spore pallet.

Scanning Electron Microscopy (*SEM*):

For SEM, the infected organs were homogenized and the homogenate was centrifuged at 6000 rpm for 10 min. After centrifugation, the pellets were collected and fixed in 2.5% glutaraldehyde for 6 h at 4℃. After fixation, washing was done with PBS buffer three times and post-fixation was done in osmium tetra-oxide for 2 h. Washing was done three times with the same buffer at 4˚C. Sample was dehydrated in acetone with 30%, 50%, 70%, 90%, 95% 100% and finally air dried with critical point and mounted on the aluminum stubs with carbon tape. The coating was done with the sputter coater and samples were observed under Scanning Electron Microscopy (Jeol, Japan; JSM 6490 LV).

Results and Discussion

Food diversification is necessary to ensure food security of large population. Fishes are crucial source of protein, vitamins and mineral. So causative parasites and other diseases needs to be explored, responsible to decline their population. One major reason responsible for fishes' decline is a microscopic, unicellular, obligate parasite-Microsporidia that are spore forming endoparasites and infect vertebrates as well as invertebrates'. Our study is in support with similar findings to explore more about this parasite. In our current study we have observed microsporidian infection in *Labeo rohita.*

Scanning Electron Microscopy was conducted to detect the morphology of spore. Ovo-cylindrical spores were detected in the homogenates that accurately exposed the structure of microsporidian spore. Ishihara (1969) isolated microsporidia that were multiplying in host cells in the form of meronts and sporonts and had a life cycle partaking only a single sporulation sequence in only one host individual.

Our study suggested the same kind of ultrastructural properties studied so far. Figure 2 denotes results of the scanning electron microscopy and revealed that spore are oval and in shape and size varies between 5.60 µm to 8.28 µm. Ovo-cylindrical spores were seen in the homogenates that accurately exposed the structure of microsporidian spore. The life cycle involves the spherical binucleated meronts to undergo binary fission and produce four nuclei per celled condition i.e. tetra nucleated meront that further forms sporonts. These forms are achieved by repetitive karyokinesis and no cytokinesis.

The study of morphological features of fish may provide additional insights into taxonomy of the parasite as well as provide information for further control of microsporidian parasite. Data of our current study suggests that microsporidian parasite has a crucial role in the pathogenesis of host. Findings of current study advance our understanding of microsporidian induced infection in fish species and will be beneficial to combat microsporidian infection in the selected fish and thereby boost its production in culture systems.

Due to its deleterious effects on fish populations, studies on microsporidian infection in fish have been conducted across the globe since

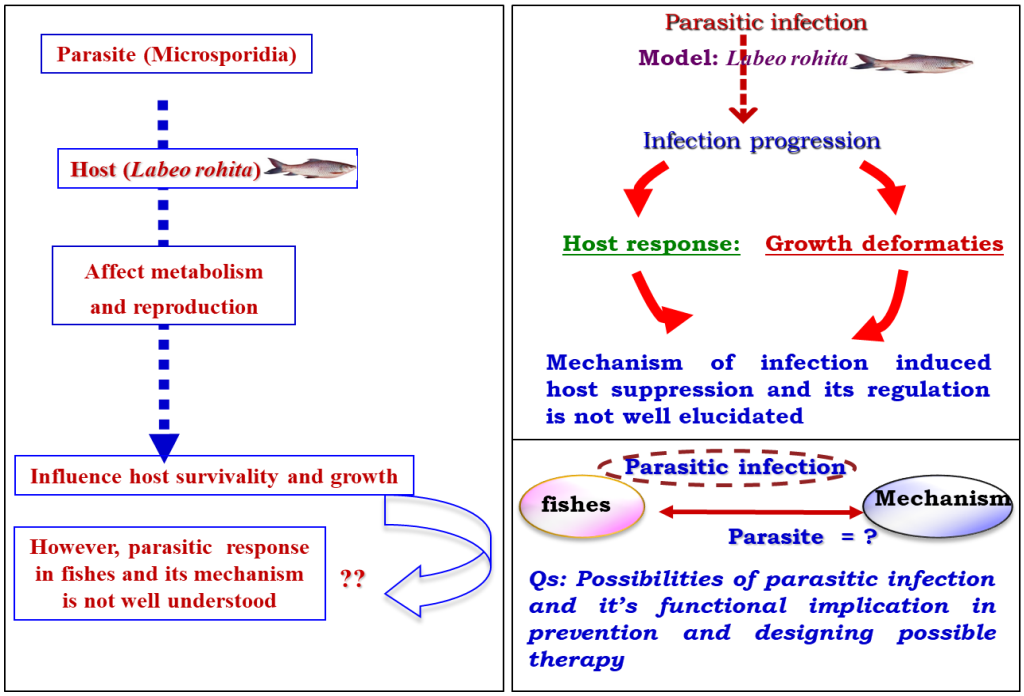

Fig. 1: Schematic flow showing hypothesis, parasitic infection and key question.

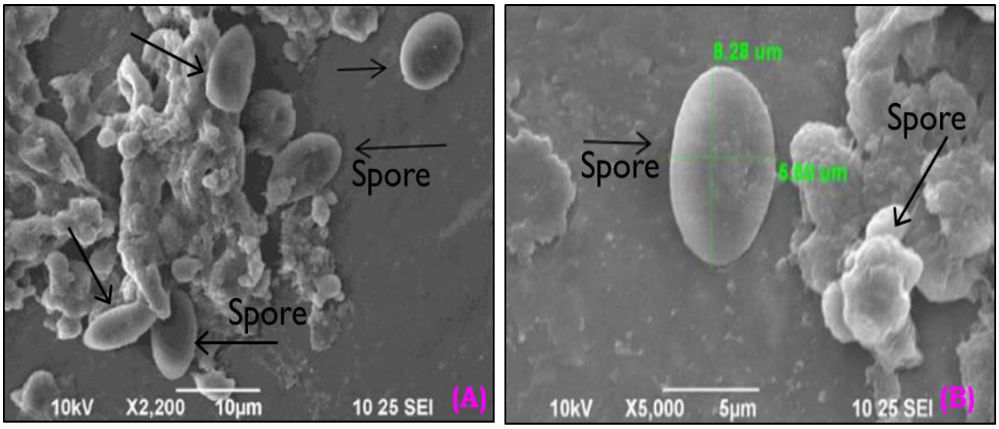

Fig. 2: Scanning electron microscopy images of spore of microsporidia.

long time. More elucidations, molecular evidences, experimental studies and advanced studies are needed to elaborate further crucial insights in understanding the course of microsporidian infection, and its effects on the host fish.

Conclusion

After detailed examination of Ultrastructural properties of microsporidia by scanning electron microscopy, the inter-relationship between the species and genus *Labeo rohita* could be established. The spores were visualized by using Scanning electron microscopy for *Labeo rohita* however, the genus cannot be identified. Without molecular study, it cannot be surely said and has to be explored more in future. Findings of this study improve our insights of the responses of the microsporidian parasite infection in fish and will be fruitful for the development of novel control strategies that reverse the parasitic alterations. Earlier high mortality was reported due to microsporidian parasitic infection by Tomamichel *et al.* (2018) hence appropriate and timely steps should be taken to overcome parasitic infection from microsporidian and enhance productivity of fish industry.

Acknowledgements

The authors extend thanks to Council of Scientific and Industrial Research (CSIR) for providing funds to support the work through a Senior Research Fellowship program, Hon'ble Vice Chancellor Professor Sanjay Singh of Babasaheb Bhimrao Ambedkar University (Lucknow), and Laboratory of Parasitology for providing the laboratory facilities to accomplish the work and USIC of Babasaheb Bhimrao Ambedkar University.

References

Boucias DG and Pendland JC. (1998) Phylum microsporidia. In: Principles of Insect Pathology. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-4915-4_12.

Casal G, Matos E, Teles-Grilo ML and Azevedo C. (2008) A new microsporidian parasite, *Potaspora morhaphis* n. gen., n. sp. (Microsporidia) infecting the teleostean fish, *Potamorhaphis guianensis* from the River Amazon. Morphological, ultrastructural and molecular characterization. Parasitology 135(9): 1053-1064.

Dey RK and Kumar D. (1986) Histopathology of microsporidia infection in Indian major carp, *Labeo rohita* (hamilton). Int Symp Aquaculture and related species held at Paris, pp. 2-5.

Didier ES, Stovall ME, Green LC, Brindley PJ, Sestak K and Didier PJ. (2004) Epidemiology of microsporidiosis: sources and modes of transmission. Vet Parasitol. 126(1-2): 145-166.

Grabda J. (1978) Studies on parasitic infestation of blue whiting (*Micromesistius* sp.) with respect to the fish utilisation for consumption. Acta Ichthyologica Piscatoria 8(1): 29-41.

Ishihara R. (1969) The life cycle of *Nosema bombycis* as revealed in tissue culture cells of *Bombyx mori*. J Invertebrate Pathol. 14(3): 316-320.

Joh SJ, Kwon YK, Kim MC, Kim MJ, Kwon HM, Park JW, Kwon JH and Kim JH. (2007) *Heterosporis anguillarum* infections in farm cultured eels (*Anguilla japonica*) in Korea. J Vet Sci. 8(2): 147-149.

Keeling PJ. (2009) Five questions about microsporidia. PLoS Pathogens 5(9): p.e1000489.

Keeling PJ. (2010) The endosymbiotic origin, diversification and fate of plastids. Philosophical Transact Royal Soc B Biol Sci. 365(1541): 729-748.

Kent ML and Stewart BJK. (2003) Transmission and tissue distribution of *Pseudoloma neurophilia* (Microsporidia) of zebrafish, *Danio rerio* (Hamilton). J Fish Diseases 26(7): 423-426.

Kummari S, Haridas DV, Handique S, Peter S, Chau CG, Sneha KG and Pillai D. (2018) Incidence of hepatopancreatic microsporidiasis, by *Enterocytozoon hepatopenaei* (EHP) in *Penaeus vannamei* culture in Nellore District, Andhra Pradesh, India and the role of management in its prevention and transmission. Int J Curr Microbiol App Sci. 7(2): 2125-2134.

Lom J and Nilsen F. (2003) Fish microsporidia: fine structural diversity and phylogeny. Int J Parasitol. 33(2): 107-127.

Lom J, Noga EJ and Dyková I. (1995) Occurrence of a microsporean with characteristics of *Glugea anomala* in ornamental fish of the family Cyprinodontidae. Dis Aquat Organ. 21(2): 239-242.

Matos E, Corral L and Azevedo C. (2003) Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporidia) and extrusion of the polar tube during autoinfection. Dis Aquat Organ. 54(3): 203-207.

McGourty KR, Kinziger AP, Hendrickson GL, Goldsmith G H, Casal G and Azevedo C. (2007) A new microsporidian infecting the musculature of the endangered tidewater goby (Gobiidae). J Parasitol. 93(3): 655-660.

Morsy K, Abdel Rahman B, Abdel-Ghaffar F and Saleh AlQ. (2013) Morphological and phylogenetic description of a new xenoma-inducing microsporidian, *Microsporidium aurata* nov. sp., parasite of the gilthead seabream *Sparus aurata* from the Red Sea. Parasitol Res. 112(11): 3905-3915.

Mosquera J, De Castro M and Gomez-Gesteira M. (2003) Parasites as biological tags of fish populations: advantages and limitations. Comments Theoretical Biol. 8(1): 69-91.

Sanders JL, Watral V and Kent ML. (2012) Microsporidiosis in zebrafish research facilities. ILAR J. 53(2): 106-113.

Sprague V, Becnel JJ and Hazard EI. (1992) Taxonomy of phylum Microspora. Critical Rev Microbiol. 18(5-6): 285-395.

Tomamichel MM, Hodgins NC, Venturelli PA and Phelps NB. (2018) The prevalence and potential fisheries consequences of *Heterosporis sutherlandae* in a Minnesota lake. Plos One 13(6): p.e0199580.