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Measurement of Infection Intensity of Microsporidian Parasite in *Labeo rohita* in Lucknow (Uttar Pradesh, India)

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Abstract: Microsporidia are eukaryotic obligate intracellular protozoan parasites reclassified as fungi, parasitizing a broad variety of invertebrate and vertebrate hosts like insects, fishes, birds, and mammals including humans. They are also responsible for major economic loss in economically important fishes. Present study was conducted on *Labeo rohita*, a well-known species of fish from carp family. *Labeo rohita* were collected from different regions of Lucknow and examined for the presence of Microsporidian infection. The present study revealed that microsporidian infection in males is more around (36%) compared to that of female (28.25%) *Labeo rohita*. Possible reason of more infection in males is due to immune system difference, hormonal factors and difference in behavioral pattern between male and female *Labeo rohita.* Also, this study revealed that microsporidian infection in Monsoon season is more around (39%) compared to that of winter season (32.25%). The actual prevalence and severity of microsporidiosis can vary depending on the specific species of microsporidia, geographic location, host populations, and other factors. Further research is necessary to gain a more comprehensive understanding of the relationship between microsporidian infections and sex differences. 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, Fish Pathogen, Light microscopy, Giemsa staining, *Labeo rohita*

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Introduction

India being the second most densely populated nation, food security is the major issue to support the health of its people. Fishing and fish farming have a predominant role in the country's GDP (Sahoo *et al.*, 2023). India contribute 7.56% of global fish production and stands second in fisheries sector after China, imparts 1.24% to the country's GVA. Fishes are well known for their high-quality proteins and fatty acids, especially long chain polyunsaturated fatty acids and certain micronutrients. Compared to other terrestrial animal food sources these components are significantly higher (in terms of quantity) in fish species. Based on such studies and evidences, FAO expert group concluded that consumption of fish is beneficial and healthy for growth and development of individual. Moderate amount of fish consumption of certain fishes (especially fatty fishes) is beneficial to reduce the risk of heart related disorders (FAO–WHO, 2011). In order to obtain sufficient amount of LCPUFAs, individuals are encouraged to increase consumption of fishes (especially fatty acid) two to three-fold times (Surette, 2008; Jenkins *et al.*, 2009; FAO–WHO, 2011). Fishes are considered as a vital source of vitamins, animal proteins and minerals. Mineral including iron, magnesium, potassium, calcium, sodium, phosphorus and iodine are essential mineral components and crucial for human nutrition (Sikorski *et al.*, 1994).

Parasites are well known for their harmful and deleterious effect on the host and modulate their physiological and biological system. Infection which is caused by viruses, bacteria and parasites in natural and man-made culture system are deleterious for health and growth of the host species. The growth of parasites on host depends on many environmental factors like seasons, habitat location, temperature and its physio-chemical properties. These factors are responsible for flourishment of different species, intensity and frequency.

Microsporidia are single-celled, obligate intracellular eukaryotic parasites that infect almost all invertebrate and vertebrate hosts including insects, fish, prawns, birds, and mammals. Historically, Microsporidia in invertebrates were first recognized as the cause of pébrine (pepper) disease in silkworms in 1857 by Carl Wilhelm Von Nägeli which severely affected the silkworm industry (Fig. 1) in France and Italy during the mid-17th century. Since then they have caused significant economic loss in the honeybee, fish, prawn and mink industries (Stentiford *et al.*, 2013)**.** The three-spined stickleback (*Gasterosteus aculeatus*) fish was the first vertebrate host in which microsporidia infection (*Glugea anomala*) was recorded in 1938 by Glugea which caused subcutaneous cysts in the host (Canning and Lom, 1986). Presently 1,500 species in about 200 genera have been reported and new species are continuously discovered in different host groups (Vávra and Lukeš, 2013 ; Cali *et al.*, 2017) .

In humans microsporidia have been considered opportunistic parasites in patients with immunodeficiency like AIDS, organ transplantation, elders, children and those patients using immunosuppressive drugs and infection also reported in immunocompetent patients (Didier and Weiss, 2011). Presently 17 species of microsporidia in 9 genera have been discovered in humans and infection has been identified in different organs like liver, skin, kidney, intestine, cornea, brain and other organs which causes even death of the patient on extreme situation (Didier and Weiss, 2006; Fayer and Santin, 2014). In fishes about 160 species of microsporidia in 21 genera has been identified (Lom and Nilsen, 2003; Stentiford *et al.*, 2013; Casal *et al.*, 2016) in ornamental, food, wild, and experimental models causing infection in gills, liver, kidney, skin, reproductive organs, resulted into reduced growth, increased mortality and loss of production.

Microsporidia contained a unique apparatus, polar tube which help to invade the host cell. The life cycle of microsporidia can be divided into three stages-- (i) the infective stages in which parasite invade the host cell cytoplasm and injects its sporoplasm through the polar tube; (ii) proliferative stage in which sporoplasm undergo merogony produces meronts; and (iii) sporogonic stage in which the meronts produces sporoblasts and then spores which are released after bursting of the cell and thus transmit the infection (Weiss, 2001; Keeling and Fast, 2002; Franzen, 2008). They are transmitted by both methods as horizontally by faecal-oral root or eating the

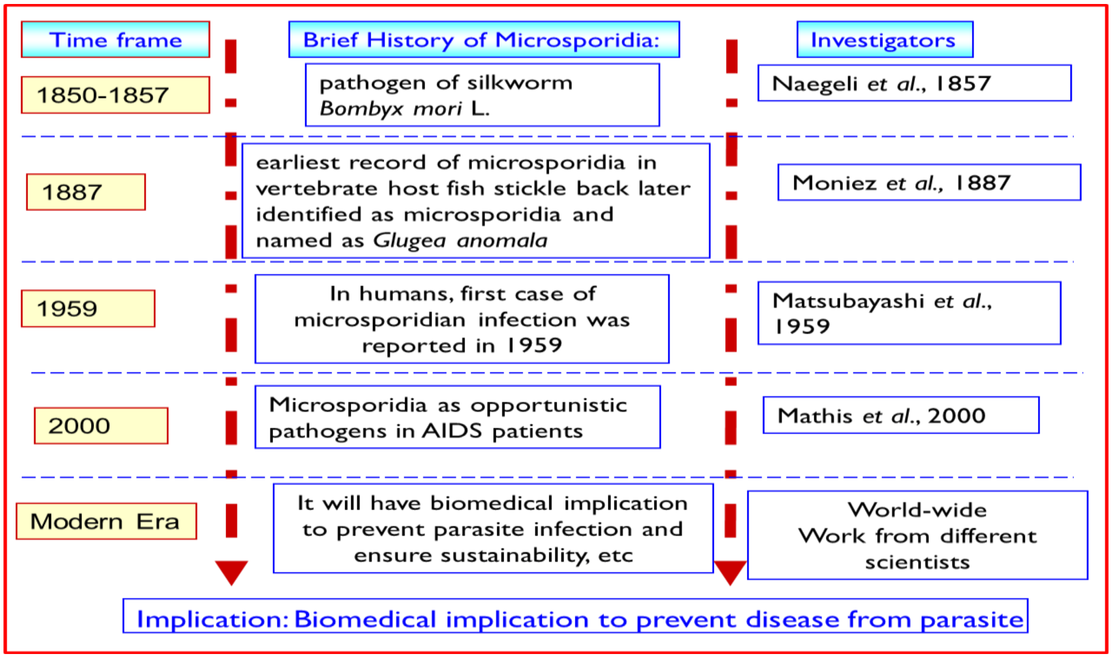

Fig. 1: Brief Historical Development in microsporidian research.

infected flesh or vertically through transovarian transmission (Dunn and Smith, 2001) in different host groups. Microsporidia are highly specialized for its intracellular mode of parasitic nature because they contain genes which are required for glycolysis, trehalose metabolism and pentose-phosphate pathway while they do not contain genes for enzyme of tricorboxylic acid and oxidative pathways so glycolysis might provide energetic molecules and other metabolites that are required for its diverse metabolic pathways to conduct different physiological processes of this organism.

Labeo rohita being Indo-Gangetic riverine species is the natural inhabitant of northern and central India, and the rivers of Pakistan, Bangladesh and Myanmar. Its 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 being parasitic host harbors also like other fishes hosts a variety of parasites, like protozoa, cestodes, trematodes, acanthocephalans, crustaceans etc. Types of ectoparasites generally found in current host fish under study are protozoan like ciliates, flagellates, myxozoans, crustaceans like copepods, helminthes like monogean, digenean etc. Common ectoparasite found in cyprinid fish under study are Protozoan parasites which are the most commonly encountered parasite in fishes. The main groups of this parasite include the ciliates, flagellates, microsporidians, ciliophora, and myxozoans. These can multiply and build up to very high numbers inside the body of host fish when fish are kept under crowded conditions, causing weight loss, debilitation of the host and ultimately leading to mortality (Klinger and Floyd, 2002).

Labeo rohita (Hamilton) is the natural diurnal inhabitant found in ponds and rivers, are bottom feeders, omnivorous fish, cannot be cultured in confined water, can tolerate wide range of temperature. *Labeo rohita* being is a bottom feeder have slightly downward mouth with thick lips, feed on Phytoplanktons, weeds, aquatic plants etc. The production from culture is badly affected by infection of parasites. Hoffman correlated the health of fishes and parasitic infestation. The parasites multiply under suitable condition of temperature, host physiology and water quality of

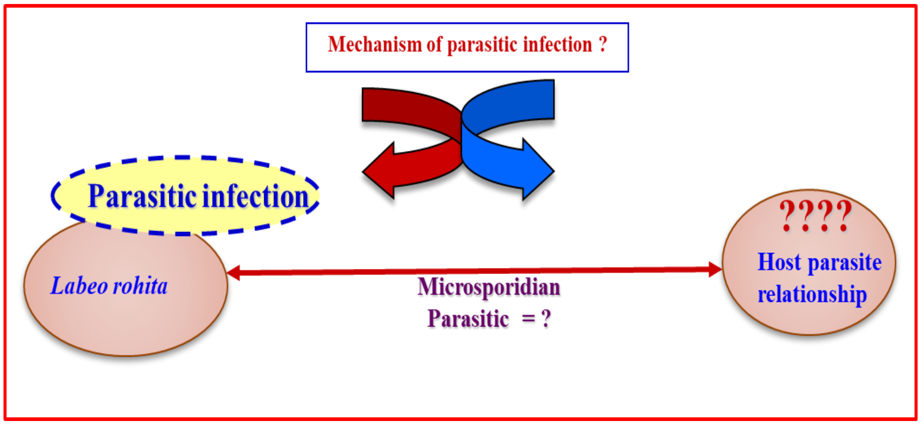

Fig. 2: Schematic flow showing unanswered mechanism of parasitic induced infection in *Labeo rohita* and its diverse host range.

the river or pond. In various findings, microsporidian infection has been reported in *Labeo rohita* (Dey *et al*., 1985).

We hypothesize (Fig. 2) that after infection, parasites influence their host in various ways. In most advanced cases the parasites rule the host cell completely and control its biological functions. Parasitic infection may influence edible fishes and may affect their survival which may have harmful effect on overall fish industry and influence aquatic ecosystem and hamper social prosperity due to diseases caused by parasites.

Present study was carried out to determine the prevalence and effects of microsporidian parasites altered infection in *Labeo rohita*. The parasites were identified by means of light Microscopy, visual inspection of infected *Labeo rohita* and Giemsa staining. The host parasite association was carried out by using several parameters. The study will give insights into the prevalence of microsporidian infection in *Labeo rohita*, a common food fish and its effects on the host. Hence, the use of *Labeo rohita* as a model to elucidate the microsporidian infection can reveal deeper insights about host parasite infection.

Materials and Methods

Sample collection:

A total of 318 *Labeo rohita* (4-30 g; 8.2-13.5 cm) were randomly collected from Aurangabad, Kalipurba, Dubbaga and Imambada regions in Lucknow through a net by the fisherman and transported to the Parasitology lab, BBAU in live condition. Fish were sacrificed and organs were collected after dissection. Fish were examined externally and whole visceral organs by naked eyes for infection symptoms.

Dissection of collected samples:

Prior to dissection the collected Labeo *rohita* were immobilized by cervical dislocation for better handling purpose. The *Labeo rohita* were dissected through the abdomen by making a longitudinal slit on ventral surface from the anus to a point level with the pectoral fins using a surgical blade or sharp pair of scissors. The alimentary canal was isolated, stretched out and grouped into intestine and gall bladder. Sections were placed in separate petri dishes containing 0.6 % saline (Kawe *et al*., 2016). Fat was removed from the intestine and then the intestine was dissected using point, sharp pair of scissors. Intestine was finally spread using the pointed forceps and brush. Thoroughly observed the spread intestine with naked eyes. Suspected specimens were collected in a separate Petri dish containing water. After collection of the suspects microsporidian parasites, they were examined under the microscope to confirm whether it is microsporidian parasite or not (Fig. 3). After confirmation Microsporidian parasites were

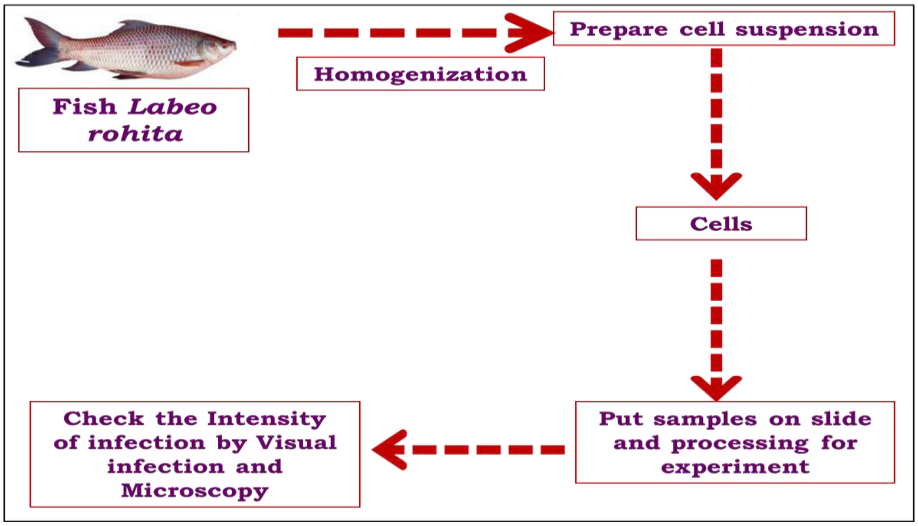

Fig. 3: Detailed Experimental methodology to detect microsporidian parasite.

processed. Confirmed microsporidian parasites were preserved by making temporary or permanent slides.

Preparation of Giemsa stain:

In 250 ml of methanol, 3.8 g of Giemsa powder was dissolved. Then the solution was heated up to 60°C on the heating mantle. Then, 250 ml of glycerin was added to the solution, slowly. The solution was then filtered and left to stand for about 1-2 months before use. The working solution of the stain was prepared by adding 10 ml of stock solution to 80 ml of distilled water and 10 ml of methanol.

Giemsa staining:

Microsporidian infected homogenate was spread on the slide and fixed with 10% methanol and dried again.Stained with dilute Giemsa stain and left overnight for proper staining. Next day it was washed by PBS and observed under live imaging microscope.

Results and Discussion

Food resources needs to be diversified to overcome the issue of food security for larger population. Fishes provides essential components of protein, vitamins and mineral. So causative parasites and diseases responsible needs to be explored, that are responsible to reduce their number.

One major causative factor to decline fish population is a microscopic, unicellular, obligate parasite- Microsporidia that are spore forming parasites and infect vertebrates as well as invertebrates (Weiss and Keohane, 1999). The continuous reduction of *Labeo rohita* populations has provoked a sense of working on reasons behind this menace. Our study is in support with similar findings with many researchers. Visual Inspection, Light Microscopy and Giemsa staining was conducted to detect the morphology of spore. Ovo-cylindrical spores were observed in the homogenates that resembles the structure of microsporidian spore (Fig. 4). 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.

The present study revealed that microsporidian infection in males is more around (36%) compared to that of female (28.25%) *Labeo rohita* as shown in Table 1 and Figure 5. Possible cause of higher infection in males is due to immune system difference, hormonal factors and difference in behavioural pattern of male and female *Labeo rohita.* Also, current study showed that

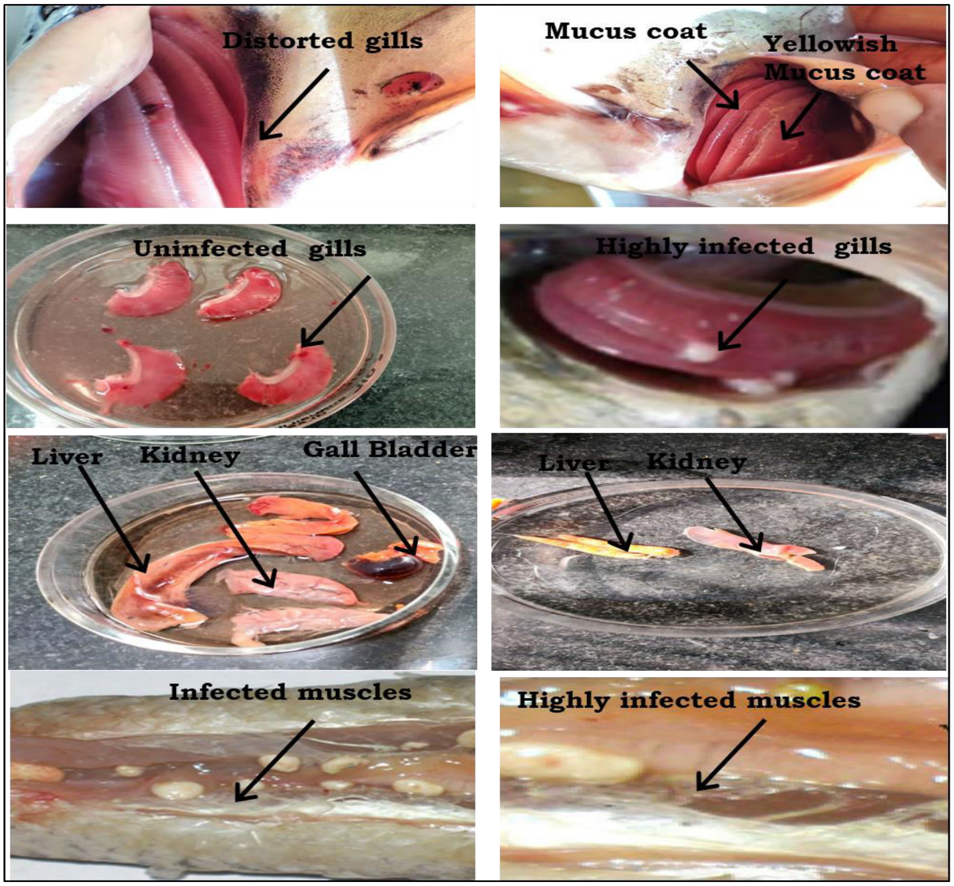

Fig. 4: Dissected organs of *Labeo rohita* for the visual inspection of microsporidian intensity.

Table 1: Microsporidian Infection Intensity observed in Female and Male Labeo *rohita*

	Aurangabad	Kalipurba	Dubbaga	Imambada	Average
Female	**25**	**28**	**38**	**22**	**28.25**
Male	**32**	**36**	**44**	**34**	**36.5**

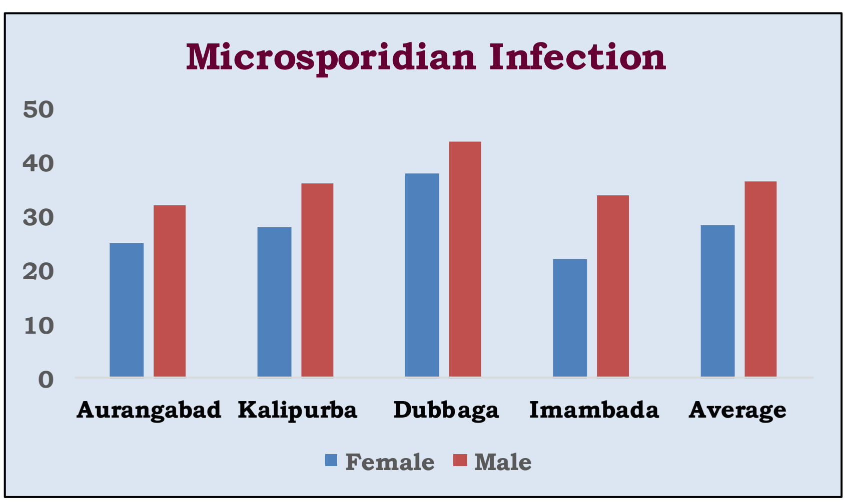

Fig. 5: Infection observed in Female and Male *Labeo rohita.*

Table 2: Seasonal (Winter and Monsoon) Microsporidian Infection Intensity observed in *Labeo rohita*

	Aurangabad	Kalipurba	Dubbaga	Imambada	Average
Winter	36	32	28	33	32.25
Monsoon	42	44	36	34	39

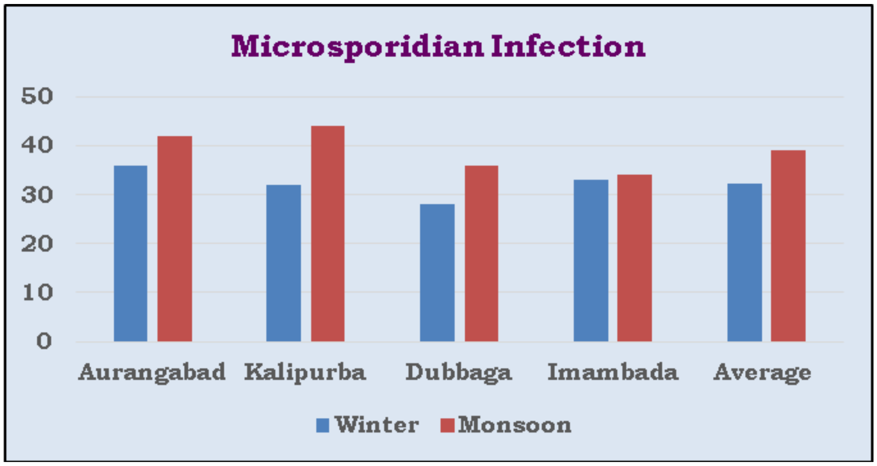

Fig. 6: Infection observed in Winter and Monsoon Season in *Labeo rohita.*

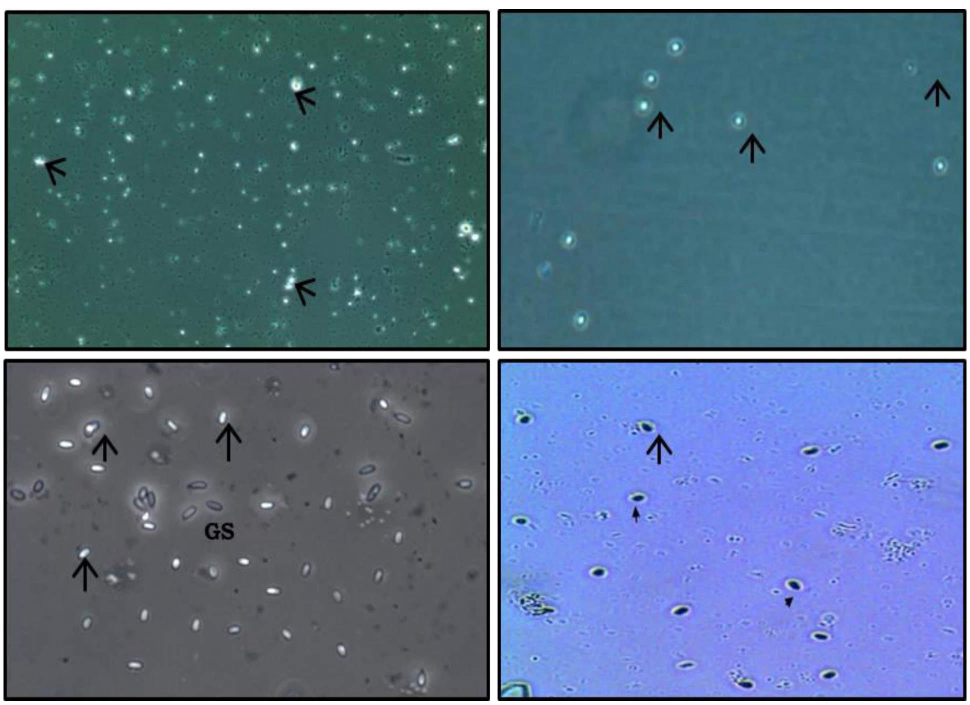

Fig. 7: Light microscopy and Giemsa stain observation of microsporidian infection intensity in *Labeo rohita.*

microsporidian infection in Monsoon season is more around (39%) as shown in Table 2 and Figure 6 compared to that of winter season (32.25%).

Our study suggested the same kind of morphological properties studied so far. Figure 7 denotes results of the light microscopy and Giemsa staining which revealed that spore are oval in shape. On light microscopic analysis, spores of two sizes were recorded i.e. 11.05± 0.27 µm ×10.15±0.61 µm (n=11) and 10.16 ±0.83 µm ×9.82±0.52 µm (n=10). The size of the spores is similar as reported previously with the size of *Ovipleistophora mirandellae* and *Pleistophora mirandellae* (7.5-12 µm and 6.5-7.5 µm) by Putz *et al*. (1965) and Lom (2002) and also slightly near the size of *Pleistophora longifilis* (macrospore= 12×6, microspore=3×2). Giemsa stained smears showed dark blue polar filament and a vacuole at the posterior side.

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.

The actual prevalence and severity of microsporidiosis can vary depending on the specific species of microsporidia, geographic location, host populations, and other factors. Due to its harmful effects on fish populations, studies on microsporidian infection in fish have been done across the globe since long time. More elucidations, molecular evidences, experimental studies and deeper studies are required to elaborate further crucial insights in understanding the course of microsporidian infection, and its overall effects on the host fish.

Conclusion

Microsporidiasis which is caused by microsporidia has been reported in this study in *Labeo rohita* collected from the different regions of Lucknow, Uttar Pradesh, India. Microsporidia damaged their host cell causing hypertrophy and liquification of the gills, gonads, and ulcers in the kidney and liver. Infection was present in all organs of fish including the brain. Microsporidia are obligate eukaryotic intracellular parasites with fungal affinities which contain a unique polar filament that imparts to infect a new host. The infected *Labeo rohita* showed discoloration of the external body part and dissolved gonads and gills with white patches on gill lamellae and whitish foci on the liver and kidney. Similar results were reported by Phelps *et al*. (2015) and Jalenques *et al*. (2021) who observed microsporidian infection-causing discoloration of muscle organs. After detail examination of Ultrastructural properties of microsporidia by light electron microscopy, the inter-relationship between the species and genus *Labeo rohita* could be established. Endoparasitic nature of microsporidian parasite is well known in fishes, but what required is the implication of this character that has to be examined further. The spores were visualized by using Light microscopy for *Labeo rohita,* however, the genus cannot be confirmed. Molecular studies need to be conducted for identification of specific species. Findings of current study improve our understanding of the responses of the microsporidian parasite infection in *Labeo rohita* and will be beneficial 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. There might be Lots of possible reasons responsible for the infection of microsporidian parasites in fishes like poor management, poor water quality, lack of quarantine measures, lack of nutrition etc. all these causative factors need to be explored because these are negatively impacting the health of the fishes. Hence, causing decline in overall productivity.

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