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IDENTIFYING CRITICAL STAGES IN THE LIFE CYCLE OF THE PARASITE *Lernaea* (LINNAEUS, 1758) BY INFECTION STUDIES IN *Poecilia reticulata*

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Abstract- *Lernaea* spp. is the most harmful ectoparasite of freshwater fishes. This study was carried out to identify critical stages in the life cycle of the fish parasite *Lernaea* sp. The experimental infection with the *in vitro* reared first copepodite stage induced infection. The results give essential insights into the pathogenic nature of the parasite and help in devising a control strategy for effectively eradicating infection and minimise economic losses to fish farmers.

Keywords- Lernaea, Guppy, Parasite, Nauplius, Copepodite, Maintenance, Infection, Fish

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Introduction

The search for effective and long-term solutions to the problems caused by parasites of fish has turned our attention to studies which help in improving our knowledge of their biology. The parasite must be available in sufficient numbers to permit study and experimentation. Experimental studies of parasite infections have proven extremely useful to our understanding of parasite ecology and epidemiology. They serve to elucidate or confirm life cycles, to measure the performance of infective stages under different conditions, or to assess the suitability of various host species for particular parasite species. Experimental studies have also been extremely useful to test the efficacy of vaccination or other treatments as protection against parasitic infection [1]. Lernaeid copepods are among the most harmful parasites of freshwater fish. The pathological changes caused by the parasite result in heavy mortality and morbidity to the fish host and thereby heavy economic losses [2].

Methods

Laboratory Maintenance of *Lernaea* on Guppy

A total of thirty guppies (*Poecilia reticulata*) were collected alive from fish farm. They were distributed in 20lt capacity partitioned glass tanks with chlorine free tap water. The water temperature was 27 ± 2°C. During this period, fish were fed daily to satiation with commercial feed.

In vitro, Rearing of the 1st Copepodid Stage

Egg sacs were removed from adult *Lernaea* females on previously infected guppies and placed in a glass beaker containing chlorine free tap water and incubated at 29°C [3]. Within, 72-80 hr post hatching, the infective Ist copepodid stage was fully developed. Each of the stages was observed and photographed under the Olympus BX53 microscope.

Experimental Infection of Guppies with *Lernaea*

Twenty apparently healthy guppies were exposed to laboratory reared 1st copepodid of *Lernaea*. Fish were immersed in water containing the infective stage (40-50 larvae / fish) for approximately 5 min. [3]. The remaining post exposure water was then emptied into the holding tank. Ten experimental fish were left without infecting as control in another tank.

Clinical Examinations

The experimental Guppies were individually inspected for detection of any gross lesions and / or visible parasitic copepod.

Histopathological Examination

The skin and underlying muscle tissue, liver of five control and five infected guppies were preserved in 10% buffered formalin. These were then embedded in paraffin and 5µm thin sections were cut in a microtome. Good sections were stained with haematoxylin and eosin (H &E).

Results

Lernaea eggs [Fig-1](a) hatched into free living naupliar stages [Fig-1](b-d) and then into the first copepodite stage [Fig-1](e). Beyond this stage the copepodites died if they could not find a suitable host. Therefore the first Copepodite stage is the first infective stage. Five more molts occur. The copepodite stages following the free living first stage move continuously on the fish host feeding on the mucus. Two or 3 days after introduction of the parasitic stages, the fish

usually show lack of mobility, folding of fins while staying close to the bottom of the aquaria. All the stages of the parasite are mobile and can be transferred between hosts by cohabitation or by manual transfer. The stage fifth copepodites are also free living. Mating takes place during this last copepodid stage. The male dies on copulation [Fig-1](f). Only the female parasites penetrate the host [Fig-1](g-i). They may either attach to the same host or move to a different one. There were signs of reddening on the fish following penetration by the stage v female copepodite. In guppies base of fins were found to be the preferential site of attachment. Once attached, they undergo metamorphosis into the adult female [Fig-1](j). The characteristic anchors keep the parasite affixed to its host. Under good conditions one female will produce several pairs of egg sacs [Fig-1](k). The experimental fishes got heavily infected at the base of the fins [Fig-1](l-n). The histological sections through the musculature of infected guppy revealed the zone of inflammation and degenerating muscle tissue surrounding the embedded anchor of the parasite [Fig-2]. The liver showed vacuolation and presence of melanomacrophage centers [Fig-3].

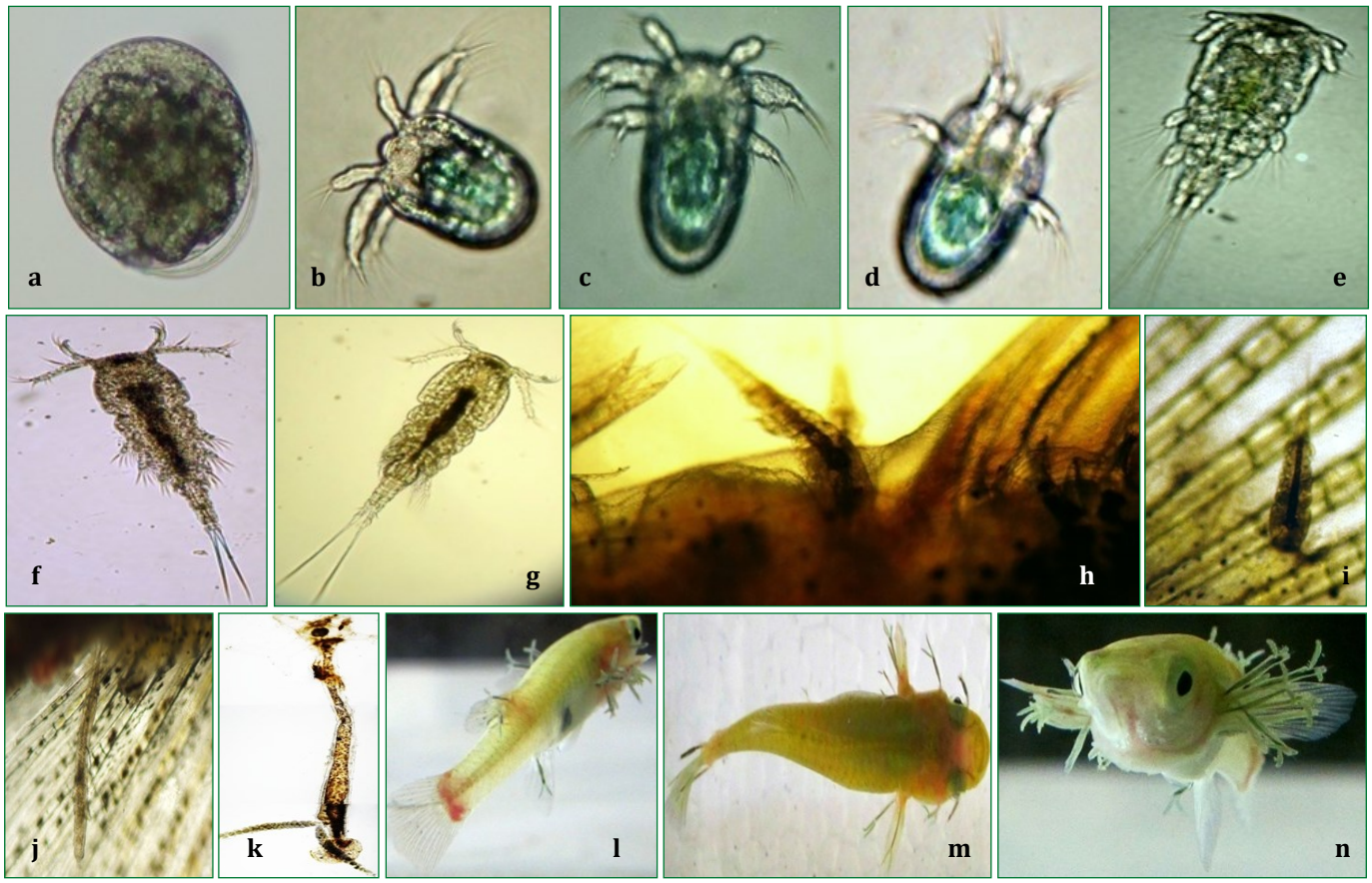

Fig. 1- a: *Lernaea* egg; **b:** Stage 1 nauplii; **c:** Stage 2 nauplii; **d:** Stage 3 nauplii; **e:** Copepodite1; **f:** Male copepodite; **g:** Female copepodite; **h:** Penetration of female copepodite at the base of anal fin of guppy; **i:**Female copepodite on fins of guppy; **j:** Metamorphosing female *Lernaea*; **k:** Adult female; **l,m,n:** Heavily infected female guppy

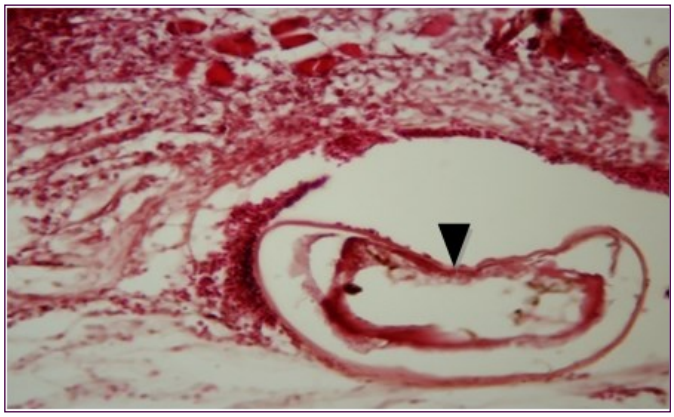

Fig. 2- Cross section of infected guppy muscle. Anchor shown by arrow head

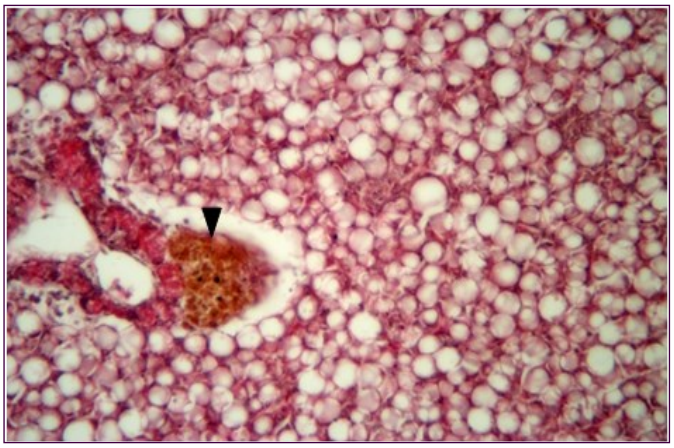

Fig. 3- Cross section through liver of infected guppy

Discussion

Fish parasite cultures in the laboratory constitute an important aspect of research on control of these organisms. Fortunately, the procedures for laboratory rearing of the most ubiquitous species, *Lernaea cyprinacea,* were previously described [3]. However; they used gold fish as a model for laboratory maintenance. Gold fish can feed on the protruding anchor worms of other fish if maintained together and thus disturbs experimental infection.

Induction of lernaeosis is important when attempting to develop useful effective protective and / or curative measures for lernaeosis. Laboratory maintenance of *Lernaea* is very tedious as the parasite is often lost from the fishes. The problems involved could be ascribed to immunity against the parasite, water quality, and parasite senescence. Fish mortality occurs as a reult of blood loss and due to the secondary infections from fungi and bacteria [4].

Female Guppy serves as an excellent model/host for the study of lernaeosis; its transparent body enables the visualisation of the copepodite attachment, movement on its surface; Ease of handling due to its small size; Easy maintenance and feeding; Does not need stringent water quality management compared to other hosts; Fish can be bred easily and in large numbers and is therefore cost effective; do not feed on the *Lernaea* on other fishes during co inhabitation.

Host size is an important determinant of the structure of ectoparasite assemblages [5, 6]. In fish populations, parasite abundance [7] and intensity of infection [8] of metazoan parasites increase with the age or size of the host. Older fish have longer body to accumulate parasites than have younger ones. Being larger, they provide more internal and external space for parasite establishment, and they have higher infection rates because they offer a larger contact area for parasite attachment [9]. However, in the present study guppies were severely infected with *Lernaea* sp. inspite of their smaller size. Even the newly released fry from the live bearers were found to be infected if the water contained the infective stages. Thus the rate of infection is not subject to size or age constraints in guppy *Poecilia reticulata*.

The first copepodite stage is the primary infective stage; the sole purpose of this free-living form is to locate and attach to a suitable host. The infective copepodid larva is typically small, but the ponds are enormous in area, potential hosts are patchily distributed and many of them are highly mobile. Clearly, overcoming the problems of locating a host and successfully infecting it are critical to the completion of the life cycle in parasitic copepods.

The second critical phase is the blood feeding adult parasitic female stage. The mature parasite is more harmful as they attack the body surface of the fish with its anchor [10] and penetrate the internal tissue of the fish after eating its scales [11]. It also ceases the feeding and reproductive activities (spawning) of the fish [12]. Furthermore, lernaeids have been reported as vectors of many pathogens such as bacteria and fungi [13]. The pathological changes caused by the parasite result in heavy mortality and morbidity in the fish host and thereby heavy economic losses.

Earlier histological studies on gold fishes infected with *Lernaea* revealed the presence of necrotized muscle cells and infiltration by inflammatory cells, including macrophages and lymphocytes [14]. Similar deleterious effects were observed on the muscle tissues of laboratory infected grass carp [15].The histopathological changes observed in the liver of *Lernaea* infected carp fingerlings involved cloudy swelling, granulation and vacuolation in the hepatocytes [16].

The present investigation was carried out to facilitate further studies that provide new information on the biology of these parasites. The ultimate anti *Lernaea* vaccine would be one that targets both the first copepodite stage and adult stage, assaulting the parasite at both major developmental stages.

Conclusion

Fundamental to the discovery of vaccines is an understanding of the biology of parasites. Different treatments target different stages of *Lernaea*, and the stage targeted may influence the efficacy of a treatment. Identifying critical stages in the life cycle of parasites may be helpful in assessing potential pathogenicity and in developing control strategies.

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Conflicts of Interest: None declared.

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