ABSTRACT

In the present study the Indian major carp *Catla catla* and the Peninsular carp *Labeo fimbriatus* were used as hosts for various experimental studies on *Lernaea cyprinacea*. The two carp species were found to be excellent hosts and susceptible species to *Lernaea*. Accordingly, these two carp species were utilized as hosts for maintaining *Lernaea* infection in the laboratory throughout the study period. Culturing the parasite under laboratory conditions at 28° C resulted in successful hatching of the parasite egg into naupliar and copepodid stages. Attempts were made to arrest further development of the copepodid-I and its prolonged maintenance under different conditions (at 4° C and 28° C) by supplementing the culture media with fish sera or liver extract. However, there seems to be no beneficial effect from supplementing the cultures with fish serum or liver extract as far as the survival of the copepodid-I is concerned, indicating that nutrients made available through the serum and liver extracts were not sufficient to create congenial conditions for increasing the time period for which *Lernaea* developmental stages can be maintained without losing its viability. Another study was conducted to establish the optimal dose of infective stages required to develop infection in fishes with minimal or no mortality of the host as well as to develop sufficient quantum of infection for further studies. *L. fimbriatus* fingerlings were exposed to copepodid-I stage at the rate of 20, 40, 60, 70, 80 and 100/fish. Infecting the carps at the rate of 70 copepodids per fish was found most suitable for maintenance of infection. In a laboratory study of biochemical changes during the development of the parasite, the soluble proteins of *Lernaea* eggs, nauplii, copepodid and adult
parasite were extracted with the ammonium sulphate fractionation (80%) procedure and the protein samples were run on 10% SDS PAGE. The electrophoretic separation reveals that *Lernaea* egg has 12 and nauplii has 6 protein bands, whereas copepodid has 8 and whole body has 13 protein bands. The high molecular weight proteins viz; 202, 169, 122, 108 and 106 kDa of the egg stage became complex proteins at the stages of nauplii and copepodid and are conserved. Proteins of 286 and 68 kDa are common for both nauplii and copepodid-I stages. However, none of the proteins from these two stages were shared by the adult parasite, indicating a complete change in the protein profile upon metamorphosing into the adult, which remains deeply embedded in the host tissue.

Studies were undertaken on the host parasite interaction and biochemical changes during experimental and natural infection of *Lernaea* in carps. Tissue from the site of parasite attachment on day 5 and 18 post infection were collected and protein was extracted. The protein samples were run on 12% SDS PAGE. No difference in the molecular weight and protein pattern from tissue samples of infected (day 5 PI) and uninfected fishes was observed. This indicated that the initial damage caused by the parasite at tissue level during the early phase of infection is minimal. During later stage of infection on day 18 PI, the protein bands observable under electrophoresis were reduced in number in tissue samples from infected fishes as compared to the uninfected control. This may be due to the lytic action of the substances released by the parasite into the host body resulting in breaking down of the high molecular weight proteins. In another study, the electrophoretic separation of serum from *Lernaea* infected and uninfected *L.*
*fimbriatus* and *C. catla* was performed. In serum of infected fishes of both species, the number of protein bands and their intensity observable under electrophoresis were reduced which indicated the loss of serum protein due to parasitic infection. In another study on the blood biochemical parameters of *Lernaea* infected carps, lower levels of total serum proteins (*P* ≤ 0.05) and Alkaline phosphatase (*P* ≥ 0.001) were observed in infected fishes. Levels of SGPT and SGOT were increased (*P* ≤ 0.10 and *P* ≤ 0.05, respectively) in infected fishes when compared with the controls. These alterations indicated the effect of the parasite on nutritional status of the host and their immunological damage and immunological response against the parasite. Histopathological changes in *Lernaea* infected *L. fimbriatus* was studied and sections of the host tissue at the site of parasite penetration and liver of the infected host showed pathological changes indicating the effect of the parasite on the host. It is concluded that the parasite penetration may lead to secondary infection which in turn results in pathological and biochemical changes in the host.

The present study evaluated the efficacy of ammonium salts against developmental stages of *Lernaea cyprinacea* and explored the use of ammonium chloride dip bath to control infection with copepodid stages of the parasite on *Labeo fimbriatus*. Ammonium chloride was found to be effective against copepodid stages at 0.025% concentration which killed the copepodid stages within 1 min 28 sec of exposure. Dip bath treatment in 0.025% ammonium chloride solution for a period of 14 minutes effectively killed/dislodged the copepodids from the host body. No parasite developed on dip treated fishes until 60 days of the experiment. Significant variations (*P*
<0.001) were observed in plasma ammonia level of dip treated groups. However, their levels returned to near normal values within 24 hours of exposure. Less significant (P ≤ 0.10) changes in chloride level of infected group was also evident. Based on the observation it is concluded that ammonium chloride dip treatment can be used as a quarantine measure to prevent spread of _L. cyprinacea_ infection during transfer of fish from affected ponds.

In case of chemotherapeutic treatments, efficacy of doramectin and ivermectin administered via oral and parenteral routes against experimentally induced and natural infections of _Lernaea cyprinacea_ in carps was studied. Administration of doramectin incorporated in feed at 1 mg/kg b.wt, of fish for 10 days effectively controlled experimentally induced _Lernaeae_ infection in _Labeo fimbriatus_ fingerlings and natural infection of this parasite in the underyearlings of _C. catla_ and _L. fimbriatus_ within an average of 19 days post-treatment as compared to the normal course of 41 days. Intramuscular administration of doramectin at 200 μg/kg b.wt, of fish effectively removed adult _Lernaea_ infection in _L. fimbriatus_ as early as 18 days of treatment as compared to the time period of 43 days taken by the untreated fish to get rid of the infection. In all cases, doramectin did not cause any noticeable adverse reactions or toxicity to the fish host. However, ivermectin administered at the rate of 1.5 and 1 mg/kg b.wt, proved to be toxic to the fishes as evidenced by the 100% and 60% mortality respectively in these two groups on day 1. Histopathological studies on liver of drug treated fishes also confirm the same. Section of the liver showed complete evacuation of cytoplasmic contents and severe vaculation indicating the toxicity of the drug. Nuclear debris and
Congestion was also observed. Administration of ivermectin incorporated in feed at 0.5 mg/kg b.wt, of fish for 10 days effectively controlled experimentally induced Lernaea infection in Labeo fimbriatus fingerlings within an average of 28 days post-treatment as compared to the normal course of 41 days. Intramuscular administration of ivermectin at 200 μg/kg b.wt, of fish effectively removed adult Lernaea infection in L. fimbriatus as early as 21 days of treatment as compared to the time period of 43 days taken by the untreated fish to get rid of the infection. In both the cases single intramuscular administration of the drug was more effective against the parasite than its administration in multiple divided doses. Another anti parasitic chemical, deltamethrin was found toxic to the fishes at concentrations of 12.5μg/l and 6.25μg/l resulting in 100% mortality. Lower concentration of deltamethrin at the rate of 3.125μg/l was not lethal to fish hosts and removed Lernaea infecting L. fimbriatus within 16 days of continuous exposure when compared to infected control group which took 41 days to become parasite free. Finally, to find out herbal compounds effective against the parasite, the efficacy of neem against L. cyprinacea was studied. Two types of neem seed powder were prepared. These consisted of (1) dried and powdered neem seeds and (2) solvent extracted neem seed powder prepared by treating dried seed powder with petroleum ether. Again different types of extracts were prepared by separately solubilizing both types of neem powder in (i) 1% NaCl solution and (ii) water at two temperatures (28°C and 90°C). The extracts so prepared were added at varying concentrations (10, 25, 50 and 100μg/ml) to the aqueous culture media containing counted number of Lernaea eggs. Results indicated that neem seed powder treated with solvent and further
solubilized with hot water (90°C) at a concentration of 100μg/ml was found to be effective in preventing the hatching of *L. cyprinacea* eggs and their further development. Nauplii and copepod Id stages were also exposed to above mentioned neem seed extracts at a concentration of 100μg/ml. Nauplii became inactive within 2 hrs and copepods died after 35 minutes exposure to solvent treated neem powder extracted with hot water. Fishes survived for an average period of 5 hrs and 30 min in all types of neem seed extracts except neem seed extracted with water at 28°C (both solvent treated and untreated neem seed powder). The ability of neem seed extract to kill the copepodid stages of *Lernaea* can be exploited and used as a prophylactic and control measure to disrupt the developmental cycle of the parasite.