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Research Article

Survivability and Transmission rate of *Centrocestus* sp. Cercariae larvae to early fingerlings of Koi carp (*Cyprinus carpio*) and Lethal number of parasites to fry stage with site preference of infection

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ABSTRACT

The infestation of cercariae larvae of *Centrocestus* sp. make considerable impact on Koi carp culture and it is important to understand the parasite behavior in order to apply control measures. The Infested snails, *Melanoides tuberculata* by *Centrocestus* sp. were induced for shedding cercaria larvae for the estimation of larval survivability. Observations were recorded at two hourly intervals. After emerging from the snail, the cercariae lived for 26 hours by maintaining a plateau period and thereafter became immotile and died after 26-32 hours. The transmission rate of the cercaria larvae to early fingerlings of koi carps (*Cyprinus carpio*) (total length 2 cm - 2.5cm) was recorded at every 02 hours until reached to zero. The transmission rate of the cercariae to the fish host was 9.34% just after the emerging from snail and this rate was increased up to 70% after two hours and remained at the constant level until an abrupt decrease in the ability to infest after 24 hours of emergence. The estimated lethal number of parasites that infested on fish fry (total length 0.8 cm -1cm) revealed that there was a positive relationship between the cercariae concentration and the mortality of the fish. The median lethal number of parasites for fish fry was estimated as 1467cercariae/L. The results indicated that cercariae larvae of *Centrocestus* sp. not only infested on gills but under heavy exposures parasite infestation can be observed on head region, body (skin) and fins of the fish which may be one of the reasons cause for mass mortalities in the koi carp culture systems.

INTRODUCTION

In Sri Lanka, ornamental fish industry attracts attention due to the income gained from local and foreign markets. Parasitic infestations have become one of the major threats in ornamental fish culture and proper health management practices should be applied to gain maximum benefit of the industry (Thilakaratne *et al.*, 2003; Sumuduni *et al.*, 2014). Among the recorded external parasitic species of fish in Sri Lanka, metacercarial stage on gill filaments of *Centrocestus* sp. has been identified as one of the reasons for the mortality of both ornamental and food fish species. After release from the snail (first intermediate host), cercaria larva infest the second intermediate host such as fish. Non-feeding free-living cercaria enters the host by active invasion. They are equipped with limited amount of non-renewable energy, usually in the form of glycogen and once resource is depleted the death of cercariae occurs (Anderson and Whitfield 1975; Tielens, 1997). Cercaria penetrates the gill filaments and encysts as metacercaria, which subsequently limit respiratory function of the fish and could cause death of the fish under heavy infestations (Balasuriya, 1988; Velez-Hernandez *et al.*, 1988; Alcaraz *et al.*, 1999; McDermott 2000; Mitchell *et al.*, 2000).

Fry and fingerling stage stocks of different fish species are distributed from fish breeding centers to outside farmers and aquaria. However, if those stocks are infested by parasites even at the controllable level, after introducing into new stocking ponds, parasites take the opportunity to spread in new environment and continue its life cycle. Therefore, necessary control measures need to be taken to prevent the dissemination of the parasite through infested stocks.

It has been recorded that the infestation of *Centrocestus* cause considerable damage to Koi carp cultures in fish breeding centers in Sri Lanka (Balasuriya,

1988). Abundance of the parasite in the surrounding environment and their behavioral pattern directly affect on the magnitude of the infestation of the host (Komatsu *et al.*, 2014). Understanding on both parasite and host is important in order to design proper health management practices in farms.

Therefore this study focused on three objectives; firstly, to determine the survival rate of cercaria larvae in water after induced shedding, secondly, to estimate the transmission rate of the cercaria larvae on gills of early fingerling stage and finally, to determine the median lethal number of parasites infested with the most preferred site of infestation of parasite on fry stage of koi carp (*Cyprinus carpio*).

MATERIAL AND METHODS

Ethical clearance was obtained from the Institute of Biology, Sri Lanka to conduct the research (Registration No: ERC IOBSL136 11 15). The infested *Melanoides tuberculata* snails were collected from earthen ponds of ornamental fish breeding centers in Rambadagalle (GPS points: 7° 51'N; longitude-80° 50'E, North western province) and Ginigathhena (GPS points: 7° 00'N; longitude- 80° 49'E, Central province). Hundred snails were kept individually in separate beakers including 200 ml of water and exposed to sunlight to initiate the shedding of cercariae. When the turbid appearance emerges in water, a drop of water from each container was observed by the microscope to confirm the shedding of cercariae. Cercaria larvae were identified according to the published literature (Hernandez *et al.*, 2003). Strained pond water was used in all stages of the experiment.

To determine the survival rate of cercaria larvae, three containers were prepared which each of them contained 50ml of water and approximately 200 freshly emerged cercaria larvae were added to each. To determine the number of survival of cercaria larvae in each container, a drop of water was examined under the microscope and the number of live individuals was counted. This was repeated for three drops and number of mean value was calculated. This procedure was followed every two hour until the survival rate reached up to zero.

To estimate the transmission rates of the cercariae, the emerged cercariae from the snails were pooled and the suspension of the cercariae was divided into 20 containers which contained 150 cercariae per 200ml water. As soon as the suspension was prepared, an un-infested Koi carp fry (2-2.5 cm in total length obtained from fish breeding center by rearing in the cement tanks) was introduced to first container and kept for one hour and examined for infestation. This was considered as the initial data of the transmission. This procedure was then repeated every two hours using a new (un-infested) fish and a new container with suspension of cercariae until infestation rate reached up to zero. Infested numbers of cercariae in gills of the fish were counted using microscope after anesthetized of fish using MS-222 (Tricaine methanesulfonate, TMS). The transmission rate was estimated using following equation;

Transmission Rate = (Number of infested parasites /Initial number of cercariae (150)) x 100

To determine the lethal number of parasite on Koi carp fry (0.8 cm-1cm in total length), the concentration of cercariae in the suspension was estimated. To prepare the concentration series (as 0, 200, 400, 800, 1600, 3200, 6400, 12800, 25600 cercariae /L), the volume from the suspension relevant to estimated number of cercaria larvae was added to each containers and the total volume was adjusted up to 250ml with the strained pond water. Then ten fish were introduced to each container. After 24hr of introduction, the numbers of dead fish in each concentration were recorded. The spearman-kaarber method (Hamilton *et al.*, 1977) was used to estimate the median lethal number (LC_{50}) of infested cercaria larvae on fish. The site preference (skin, fins, and gills) of infestation on the host by the parasite was also examined at each concentration and recorded for the whole series. Water quality parameters were measured using test kit (API test kit, fresh water master test kit) and all parameters were maintained at constant levels in each container during the experiment (Nitrite-0.002ppm, Dissolved Oxygen-6ppm, PH-8, Ammonia-0.02ppm, Sulphide-0.00ppm, Temperature-32°C, Hardness-93ppm) and experiment was repeated three times.

RESULTS AND DISCUSSION

Generally, young Koi carps are stocked in ponds when the size ranging from 1cm - 3.5cm in length, and two length class groups within above mentioned range range were used in the current study. It has been recorded that smaller young fishes are more susceptible for parasite infestations and thus show high mortality (McDonald and Bonner 2006).

Cercaria larvae were identified using their morphological and meristic characteristics such as heart shape body, single tail, two eyespots, oral sucker etc. and confirmed as the cercarial stage of *Centrocestus* sp. Figure 1 shows that the cercariae of *Centrocestus* sp. survived well up to 26 hr after emerging from the snail and thereafter the survival declined sharply, and the mean number of live cercariae decreased to almost zero by 32hr of post emergence.

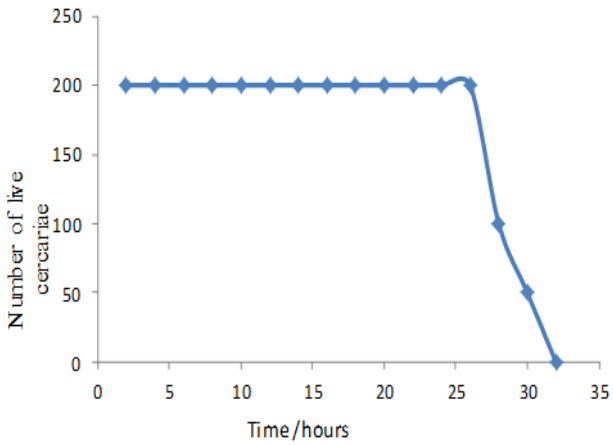


Fig. 1: Survival of *Centrocestus* sp. cercaria larvae

Results of the survivability of cercaria larvae of *Centrocestus* sp. showed slight difference when compared to *C. armatus* (Paller *et al.*, 2007); number of live cercariae decreased to zero after 32hrs from emerging for the former species while it was reported as 38hrs from emerging for the latter species.

According to data, the initial transmission rate of *Centrocestus* sp. was 9% and then it was progressively increased until attaining to the maximum value of 70% within 4 hours of exposure time (Figure 2). Transmittance

rate was then slightly fluctuated at the level of 70% for a period of 24hrs and decreased abruptly within next 8 hours.

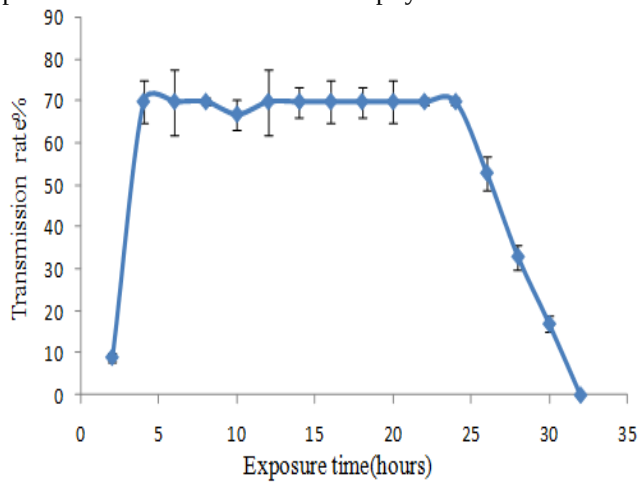
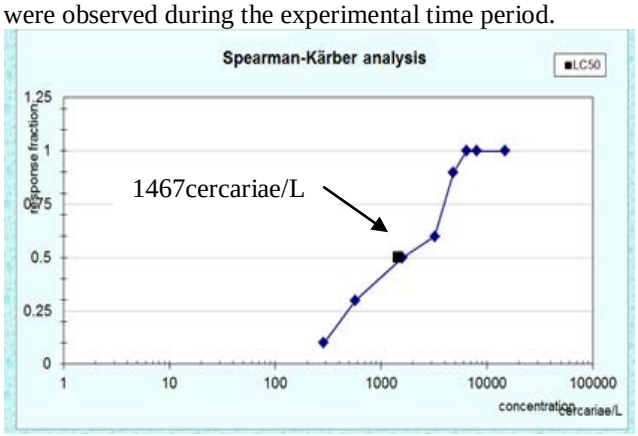


Fig. 2: Mean transmission rate of cercaria larval stage of *Centrocestus* sp. into gills of fry stage of Koi carp (exposure to concentration of 150 cercariae /L solution)
(Bars- standard Deviations)

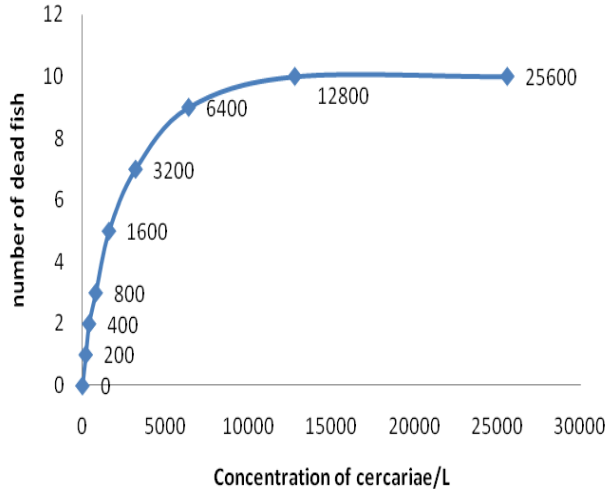
It has been reported that the glycogen amount is high within cercariae just after emergence from the snails (Lawson and Wilson, 1980; Morley, 2011) suggesting that there must be a high transmission rate at this stage. However, during the current study the parasite demonstrated a lower transmission rate. It may due to the period of acclimatization of cercariae to the environment. Transmission of cercariae to fish is a critical factor influencing dynamics of infestation. The cercariae concentrations in ponds could be varied according to the climatic conditions and the available snail density. It has been reported as cercarial shedding and infestation is a temperature dependent process (Achiorno and Martorelli 2016) and the optimum temperature for emergence of pleurolophocercous / monostome cercariae was 25°C and it starts to decrease when the temperature reaches to 30°C.

The susceptibility of a fish for a disease depends on the fish species, density, behavior etc. as well as the species of parasite and the water quality parameters especially on temperature (Morley, 2011); in the current study the optimum infestation was 70% at 32°C for *Centrocestus sp.*, while it was 87% at 20°C for *C. armatus* cercariae as recorded by Paller *et al.*, (2007).

According to the spearman-kaarber method, 50% of fish died in the concentration of 1467cercariae/L (Figure 3A) while the concentration was 12800cercariae/ L for 100% of fish mortality (Figure 3B). In controls no dead fish were observed during the experimental time period.



3(A)



3(B)

Fig. 3: **(A)** Graph of spearman karber analysis test; **(B)** Mortality of Koi carp fry related to the concentration of infection of cercariae in water

Fish fry are more susceptible for infestations compared to adults. Their gills are more delicate structures and minor damages occurred in gills make fish fry vulnerable to osmoregulatory as well as respiratory difficulties (Ellis *et al.*, 1978). Therefore, even fewer numbers of cercariae cause greater disruption in the gills of young fish (Fischer and Kelso 1988). Pauly (1981) suggested that the small fish died with the influence of fewer metacercariae than larger fish as they have less gill surface area than larger fish. In addition to that, their relatively thin skin, lack of previous infestations (Lun *et al.*, 2005), having slower defense response mechanism than larger fish (Mcdonald and Bonner 2006), differences in the age-related quantity or structure of biochemical compounds expelled by fish might be other reasons for higher infestation in small fish (Haas 1992).

In low concentrations of cercariae, only the gills of the Koi carp fry were infested. But under high concentrations, infestations can be observed in the gills, head region, body and fins (Table 1).

Table 1: Percentage of infested Cercariae of *Centrocestus* sp. on different parts of the body of Koi carp (*Cyprinus carpio*)

Cercariae Concentration (cercariae/L)	**Percentage of *Centrocestus* sp. cercariae infested on different sites of the Koi carp fry (*Cyprinus carpio*)**			
	Gills	**Head region**	**Body (skin)**	**Fins**
0	UI	UI	UI	UI
200	100	UI	UI	UI
400	100	UI	UI	UI
800	100	UI	UI	UI
1600	100	UI	UI	UI
3200	76.27	16.95	6.77	UI
6400	62.5	25	10.93	1.56
12800	48.93	26.6	21.27	3.2
25600	50	27.02	20.27	2.25

Note: UI-Un-infested

High abundance of parasites of *Centrocestus* sp. was found in the gills of the fish fry and less number of parasites found in other infested sites (Figure 4). Even under high concentrations of parasite, fins were infested in less numbers of cercariae. Therefore, it could be suggested that the gills were the most preferred site of infestation.

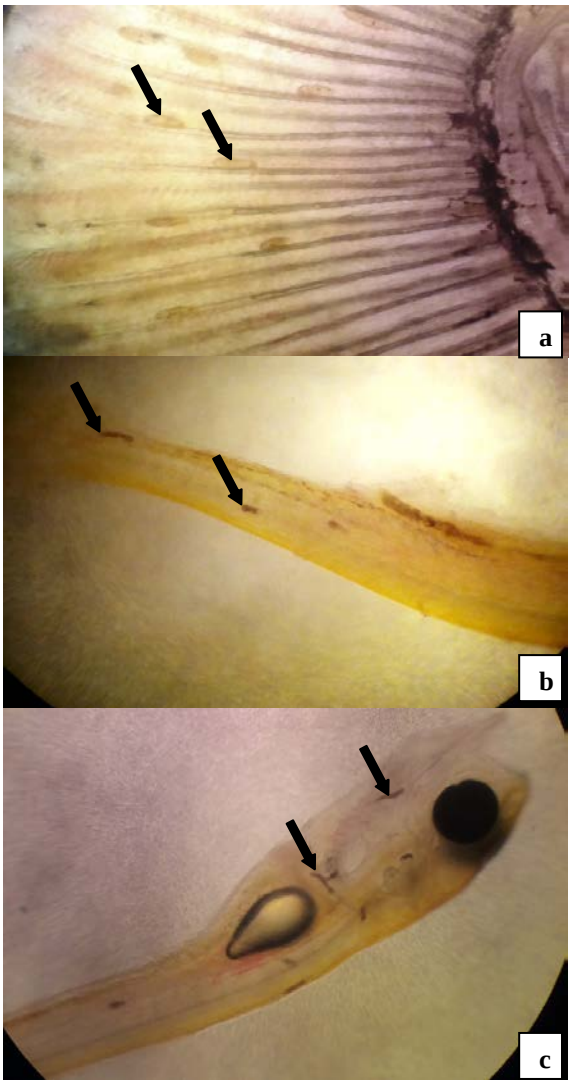


Fig. 4: Cercaria larvae of *Centrocestus* sp. infested on a. gills, b. fins and skin and the c. head region of koi carp fish fry

Current study revealed that the infestation of *Centrocestus* sp. not only confined to gills but also infest on the head, body regions and fins of fish under high concentrations of the parasite. This may be one of the reasons for the mass mortility of fish fry which is experienced in koi carp culturing systems (mud ponds). The Mcdonald and Bonner (2006) have reported that the larval fish are more susceptible to mortality induced by cercariae of *Centrocestus* sp. compared to juvenile and adult stages of fish of fountain darter fish (*Etheostoma fonticola*). According to the survival curves resulted in his study, 50% of larval fish (9-13mm total length) were died within 116 minutes, but it was spent 330 minutes to die 50% of fish with the total length of 16-20mm and 8 hours to die 25% of adult fish with total length of 36-41mm.

The infestation of *Centrocestus* is mainly reported from both food fish and ornamental fish species reared in earthen ponds in Sri Lanka (Balasuriya, 1988; Sumuduni *et al.*, 2015; Vinobaba 1991). Koi carps are stocked in earthen ponds from the age of seven days until they attain fry or fingerling stage and are then harvested for distribution to local or export markets. The fry stage of fish is more susceptible for parasites and therefore it is adversely affect on the production of the industry.

Results of this study revealed the mortality level of koi carp fry related to the concentration of cercariae in water. Therefore, continuous monitoring of water samples for parasites and control their density is important as a preventive measure. Control of snail population is the best method to minimize the parasite density in water which acts as the main distributing factor of the parasite. Removal of vegetation including both algae and vascular plants, application of approved herbicides, drying pond bottoms, and application of lime, delay culture seasons, supplemental stocking of snail predators could be suggested as best management practices to implement.

CONCLUSION

Findings of the current study enable a greater understanding of the behavior, transmission and lethal density of cercaria larvae of the parasite *Centrocestus* sp. to early fingerlings of koi carp which could be used as basic information when planning best management practices and strategies in koi carp culturing systems.

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