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Seasonal Incidence and Pathogenicity of the Metacercariae of *Clinostomum complanatum* in *Aphanius dispar*

ABDULRAHMAN M. N. KALANTAN, MISBAHUL ARFIN AND WAJIH A. NIZAMI

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Abstract

Seasonal variation in the incidence and intensity of *Clinostomum complanatum* metacercariae in relation to size, sex and region of the body of *Aphanius dispar* have been studied. The incidence varies from 8.0% to 86.6% with three peak periods during October, March and July and the intensity of infection ranges from 4 to 41 metacercariae/fish, with three peak periods during October, March and July. As a whole 47.5% male and 55.9% female fishes were found to be infected and intensity of infection was more in female than in the male fishes. The worm burden was not uniform throughout the host body; trunk was the most preferred region. The possibilities of such differences in the metacercarial population with season, size, sex and region of the host body have been discussed.

Histologically, degenerative changes in the muscle tissues around the cyst, pressure atrophy and degeneration of hepatic cords as well as cellular infiltrations in the kidney were observed, indicating the pathogenicity of the metacercariae.

Key words: *Clinostomum complanatum*, metacercaria, incidence, histopathology, Arabian barred killifish

Introduction

Progenetic metacercariae of *Clinostomum complanatum* are cosmopolitan in distribution (see references in Kagei *et al.* 1984). In Saudi Arabia, Cyprinodontid fish *Aphanius dispar* (Arabian barred killifish) is found heavily infected with this metacercaria. This fish has considerable economic value, being a forage fish for the fish culture as well as it has been reported to be a mosquito larvivorous fish in India, Saudi Arabia and elsewhere (Prashad and Hora, 1936; Jenkins, 1964; Al-Dham *et al.* 1977, and Ataur Rahman, 1981) and can be used in biological control of malaria. This is a report on the incidence and intensity of *C. complanatum* metacercariae in relation to the size, sex, region of the body and its pathogenicity. The scarcity of information on this aspect together with the importance of fish as mosquito larvivorous, emphasizes the need for this study. Further it is the first report on incidence of *C. complanatum* and its pathogenicity in *A. dispar*.

Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.
**Department of Zoology, Aligarh Muslim University, Aligarh, India.*

Materials and Methods

Aphanius dispar (Ruppell, 1828) were collected with the help of Scoopnets from the fresh water ponds in the Al-Hassa region (Lat. 25°2′N: Long. 49°34′E), Saudi Arabia, during October, 1982 to September, 1983. All fishes were transported to the laboratory alive and maintained in aerated aquaria prior to dissection and examination for encysted metacercariae. Fishes were necropsied within a week after collection and incidence of infection was recorded on the basis of presence or absence of encysted metacercariae. In order to study the incidence and intensity of infection in relation to size of the host, fishes were divided into three size groups, small (1.5–3.0 cm), medium (3.1–4.5 cm) and large (4.6–6.5 cm) long.

Table 1 Percent infection, worm burden, and distribution of the metacercariae of *Clinostomum complanatum* in different regions with respect to sex of the host

Months / Sex	No. of fishes Examined	No. of fishes Infected	Percent infection	Total worm burden	Mean worm burden/fish	Total worm burden in Head region	Total worm burden in Trunk region	Total worm burden in Tail region
October, 1982	30	26	86.7	1060	41	336	502	222
November	50	24	48.0	247	10	77	150	20
December	25	2	8.0	55	28	15	22	18
January, 1983	77	33	42.9	234	7	58	129	47
February	88	68	77.3	325	5	85	142	98
March	37	29	78.4	1046	36	355	464	227
April	40	30	75.0	256	9	85	106	65
May	50	23	46.0	184	8	49	95	40
June	70	22	31.4	132	6	34	67	31
July	65	55	84.6	1650	30	466	785	399
August	99	56	56.6	672	12	170	350	152
September	90	30	33.3	223	7	89	64	70
Male	59	28	47.5	195	7	57	92	46
Female	662	370	55.9	5889	16	1762	2784	1343
Total	721	398	55.2	6084	15	1819	2876	1389

For histopathology, the infected fish specimens were fixed in A.F.A./Bouins fluid/ Neutral buffered formalin and paraffin sections of the encysted metacercariae along with the host tissues were stained with Harris hematoxylin-eosine, Periodic acid Schiff (PAS), Mallory's Aniline Blue and Gomori's Trichrome stains (McManus and Mowry, 1960; Drury and Wallington, 1976).

Results

During the year-long survey of *Aphanius dispar,* 398 fish specimens out of 721 were found to harbour the metacercariae of *C. complanatum.* Incidence of infection varried from 8.0% to 86.6% while intensity of infection from 4–41 metacercariae/fish, but the infection was present throughout the year. Total number of hosts necropsied every month, percent infection and worm burden are given in Table 1.

Seasonal incidence and intensity of infection:

The percentage of infection and mean number of metacercariae per infected fish showed seasonal variation (Table 1). The incidence had three peaks during the year (October, March and July) whereas the intensity of infection had four peaks (October, December, March and July). The three peaks of intensity coincided with those of incidence but the fourth one obtained in the month of December is contrary to the incidence which was at the lowest (Fig. 1).

Infection rate in relation to size of the fish:

The incidence and intensity of infection were studied in relation to size of the fish.

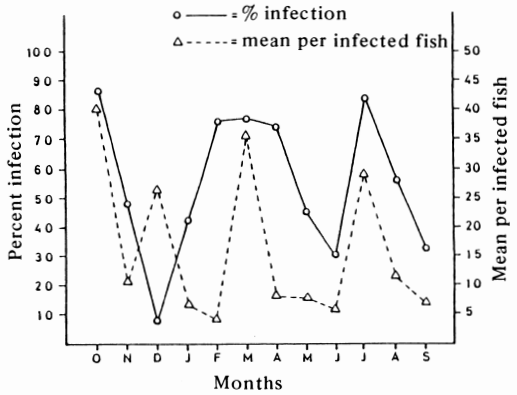

Fig. 1 Seasonal incidence and intensity of infection of the metacercariae of *C. complanatum* in *Aphanius dispar.*

There was an increase in the incidence of infection with the length of the host. As a whole 39.6%, 52.4% and 74.7% fishes of small, medium and large size groups respectively were found to be infected. The mean worm burden per infected fish in three size groups also varied and there was an increase in the mean number of metacercariae per infected fish with the increase in size of the host. The mean worm burden was 5, 10, and 27 per infected fish in small, medium and large group respectively. The incidence and intensity of infection in hosts of three size groups also showed seasonal variation (Figs. 2 and 3). The

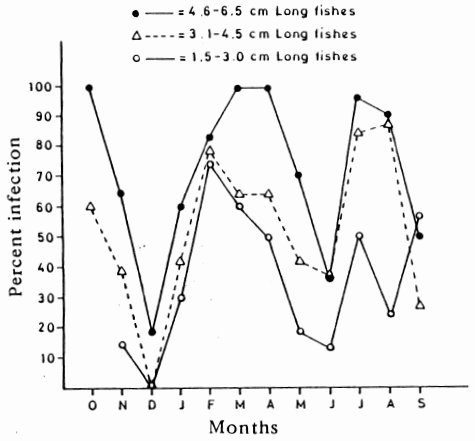

Fig. 2 Seasonal incidence of infection of the metacercariae of *C. complanatum* in relation to size of fishes.

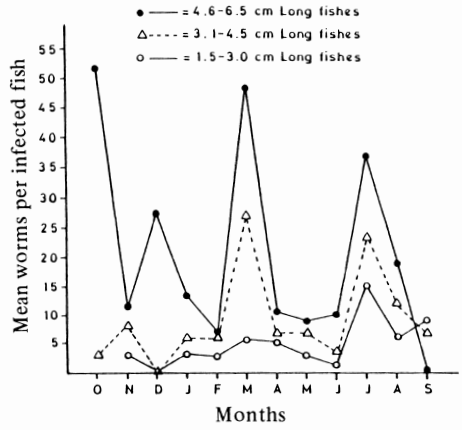

Fig. 3 Seasonal intensity of the metacercariae of *C. complanatum* in relation to size of fishes.

large size group had three peaks of incidence (October, March–April and July), the medium size group showed three peaks (October, February and August) while the small one had three peaks (February, July and September). In October no small size fish could be obtained. Although the largest size group had four peaks of intensity in October, March, December and July, the peak in December can be neglected because the incidence was the lowest as only two fishes out of twenty-five were infected and had quite good number of metacercariae (Table 1). The medium size group had three peaks of intensity in November, March and July while the smallest one showed two peaks in July and September.

Infection rate in relation to sex of the host:

The number of the male fishes is very less compared to the female fishes, constituting only about 8.0% of the total population. Therefore, the variation in the incidence and intensity of infection in relation to sex of the host with season could not be traced out. However incidence of infection as a whole was 47.5% and 55.9% in males and females respectively. Intensity of infection was more in female than in male fishes.

The mean worm burden per fish was 7 and 16 in male and female respectively and the maximum number of metacercariae obtained from a single host was 207 from 5.5 cm long female fish and 16 from 5.0 cm long male fish, both collected in March.

Worm burden in different region of the host body:

Worm burden was not uniform throughout the host body (Table 1). Intensity of worm in different body regions of the fish indicated that trunk region was the most prefered region as 47.3% of the total worm burden was found in this region while 29.9% and 22.8% in head and tail regions.

Histopathology:

Externally, the metacercariae of *C. complanatum* were found encysted under the scales, on ventral, lateral sides of the fish, around the eyes and nasal region (Photos. 1 and 2). In addition, the metacercariae were also found in the

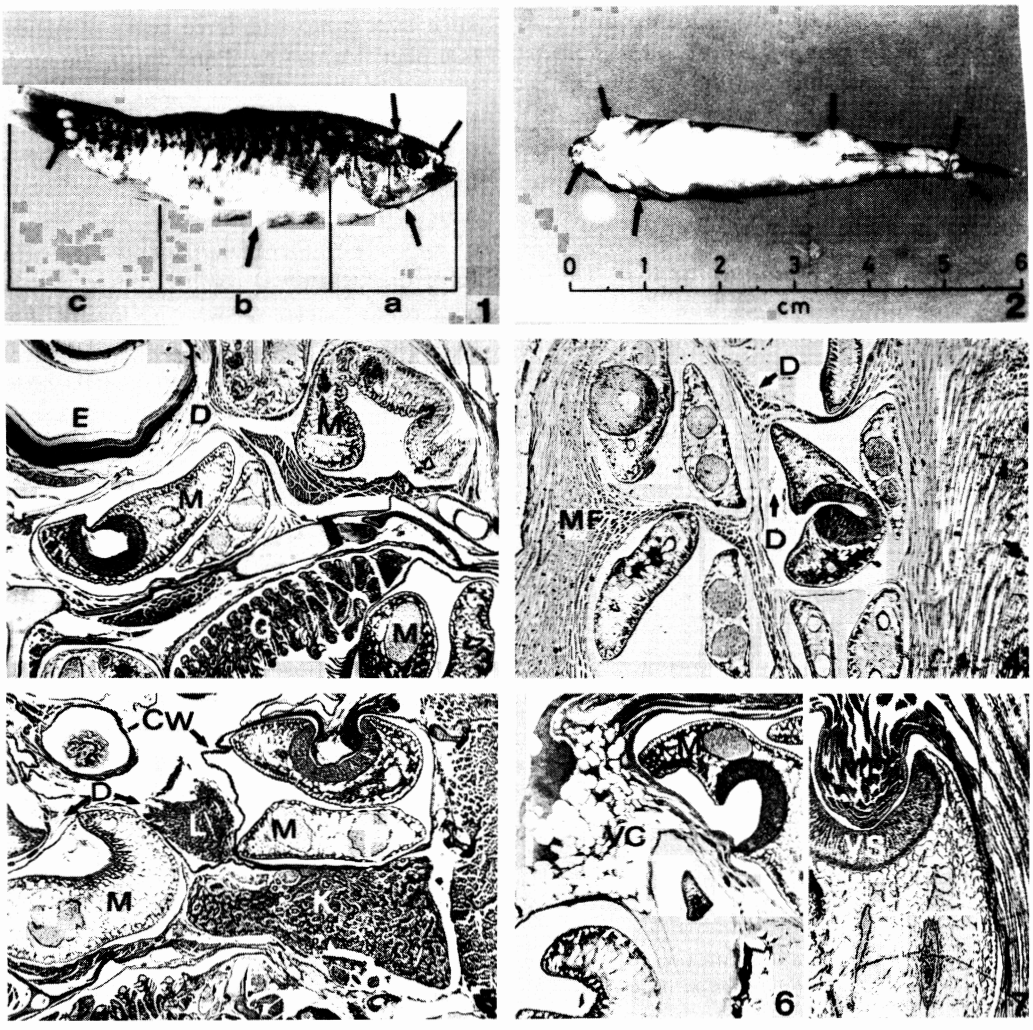

Photo. 1 Cysts of *Clinostomum complanatum* on the body of *Aphanius dispar*, shown by the arrows and conventional regions of body taken to find the worm burden in different regions. a, Head region: b, Trunk region: c, Tail region.

Photo. 2 Ventral view of fish showing encysted metacercariae.

Photo. 3 Histological section of fish showing metacercariae and degenerated cells near eye and gills.

Photo. 4 Section of fish showing metacercariae and degenerative changes in the muscle fibres of trunk region.

Photo. 5 Section showing degenerative changes in liver and kidney.

Photo. 6 Section showing large vacuolated cells filling the space vacated by necrotic muscle cells.

Photo. 7 Section showing the ventral sucker of metacercaria holding the host muscle fibres.

Abbreviations used: CW, cyst wall; D, degenerating cells; E, eye; G, gills; K, kidney; L, liver; M, metacercaria; MF, muscle fibres; VC, vacuolated cells; VS, ventral sucker.

visceral tissue and muscles. The metacercarial cyst is bilayered and both the layers are contributed by the host as a consequence of defensive reaction at the host-parasite interface (Kalantan *et al.* (1986). The presence of parasite even in non-encysted form resulted in the necrotic degeneration of surrounding muscle cells (Photos. 3, 4 and 7).

Histological degenerative changes were also evident in the muscle tissues around the cyst

and large vacuolated fat cells were usually found filling in the spaces vacated by necrotic muscle cells (Photos. 5 and 6). The blood vessels were congested and in liver, a pressure atrophy of tissue as well as degeneration of hepatic cords were noticed. Similarly in kidney together with degeneration of tissues, congregation of reactionary cells was observed.

Discussion

Incidence of infection of *C. complanatum* in *A. dispar* varied during the year. The three peaks in spring, summer and rainy season can be correlated with the availability of the snail host, environmental temperature and frequency of visit of final host to ponds.

Various reportes are available on incidence of metacercariae of *Clinostomum* spp. in other fishes from different geographical areas. Koval (1962) reported the incidence of *C. complanatum* to be 10% to 33.3%. Galieva (1971) found variations in infection rate of *Perca schrenki* by *C. complanatum* metacercariae according to season, region and fish size at the Balkash-Alakol Basin, Kazakhstan, U.S.S.R. The percentage incidence varied from 6.1 to 92.8 according to habitat, but investigations were made from May to September only. Paperna (1980) reviewed various reports on incidence in *Tilapia* spp. as 55% to 80% in Sudan Nile, 3% to 65% in Niger River at Kainji and dam reserviors in South Ghana; in Siluroid fish, 4.4 to 21% and in *Synodontis* spp. 35 to 57% in lower Niger. Siddiqui and Nizami (1982) reported the incidence of *C. complanatum* in *Trichogaster fasciatus* to be 61% in females and 63% in males from North India.

Yoshino (1940) attributed the annual fluctuations in occurrence of metacercariae of *C. complanatum* in *Carasius auratus* in Okayama Province of Japan to water temperature. Similarly, Grabda-Kazubska (1974) also correlated the occurrence of metacercariae of *C. complanatum* in Poland with the artificially heated water. Studies on seasonal occurrence of *Clinostomum* spp. among *Tilapia* spp. in Nungua Dam in South Ghana showed that the peak infection among the fishes occurred towards the end of rainy season during September–October, while prevalence of infection was at its lowest levels during dry seasons between December to May (Paperna, 1980). In present study too, the highest peak was in October which is the rainy season probably because in the rainy season the snail host (*Lymnaea stagnalis*) population also increases whereas the month of December, which is the winter season, the incidence was very low. Chubb (1979) stated that the single most significant seasonal factor in the mid-latitude climates appears to be temperature. The temperature exerts the greatest influence on the hatching of the miracidium (Dubinina, 1949), development of larvae in the snail host and their liberation (Kendall, 1965) as well as formation of metacercariae (Chubb, 1979). Further, in summer the birds are expected to visit the ponds much frequently.

Keeping in view the present results together with previous reports, it can be suggested that the environmental temperature and availability of the 1st intermediate host may be among the factors which cause the seasonal variations in the incidence and intensity of metacercariae in the fishes.

As the parasitization is not uniform through the population and suggested by Chubb (1979) that, in an ideal study of the host-parasite relationship, the fishes should be divided into age classes and length groups. Therefore, the incidence and intensity of infection were studied in relation to size of the fish. Although the length may vary according to growth rates which can change from one habitat to another (Chubb, 1979). To minimize these variations in growth rates, all fishes throughout the study were collected from same ponds (habitat). Incidence and intensity of infection varied with the size (Figs. 2 and 3). These variations in the incidence and intensity of infection with the size of the fish can be correlated with (1) the size of the surface area available for penetration and settling (2) the time duration for which the fishes are exposed to the cercariae because the size is often related with the age

of the fish and as postulated by Dogiel (1961) that, there is general increase in the intensity and incidence of infection with the age of the host. Kennedy (1975) also suggested that the older host has a longer period to make contact with the parasite. Moreover in the fishes of more than one year of ages (the large size group), accumulation of metacercariae from year to year may result in the increase in incidence and intensity of the metacercariae as these metacercariae can survive over winter, from one season of invasion to the next (Fischthal, 1949). In December, where the incidence was lowest, the occurrence of quite a good number of worms in two fishes of large size group may be explained on the same basis and these worms might have been acquired during the pervious season of invasion. Thus the incidence and intensity of infection increase with the size/age of the host.

There was a significant difference in the incidence of *C. complanatum* in the two sexes of *A. dispar* and the intensity of infection was higher in females than males. This may be due to the reason that the spawning takes place on submerged leaves and before and during spawning, the females are lithargic, so the cercariae get more chance to penetrate. Moreover, the lymnaid snails are known to be surface dweller (Hornell, 1951), so chances of cercarial contact with females during the spawning are greater than the males. Similarly, Siddiqui and Nizami (1982) related the biology of the fish *T. fasciatus* to the difference in the metacercarial population of male and female fishes.

As stated earlier, 47.3% of the worm burden was localized in the trunk region, i.e. in between the origin of pectoral fin and anus. This can be explained on the basis of the entrance of cercariae with water current through the opercula, and so it may be easier for the cercariae to penetrate the soft tissue lying under the operculum than on the scaly surface.

The stained serial sections of the infected fish showed degeneration of the muscle tissues around the cyst. Similarly, Rai (1970) recorded the degenerative changes in the muscle tissues around the cyst in three species of fishes (*Ophiocephalus punctatus, Clarias batrachus* and *Heteropneutes fossilis*). He (loc. cit) also found cellular infiltration in the connective tissues. However, the reactionary cells were less in number but the blood vessels were congested. Singh and Virmani (1978) reported anaemia in *Colisa fasciatus* infected with metacercariae of *C. piscidium.* Recently, Liu (1979) and Lo *et al.* (1979 and 1982) reported serious economic disturbances to some fish culturists in Taiwan caused by *C. complanatum.*

Conclusively it can be suggested that environmental temperature, availability of the snail host, size and biology of second intermediate host may be among the factors causing the variation in the population of metacercariae of *C. complanatum* and the metacercariae causes considerable damage to the host tissue.

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