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Gymnorhynchus gigas Plerocercoid (Cestoda: Gymnorhynchidae) Infection of the Liver of *Diodon hystrix* (Pisces: Diodontidae)

I. Incidence and Intensity of Infection, Histopathology and Effect on Hepato-Somatic Index

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The nature of infection of the liver of *Diodon hystrix* by the plerocercoid larva of *Gymnorhynchus gigas* has been studied. The parasite is both host and tissue specific and is reported for the first time from the South-west coast of India. The incidence of infection is high in colder months. Fish of intermediate size are more infected than the smaller and larger ones. Males seem to be more susceptible to infection than females. The histopathological change is in the form of a typical inflammatory reaction aimed at encapsulating the parasite in fibrous conncetive tissue cyst. The infection adversely affects the total body weight and the liver weight resulting in a conspicuous lowering of the Hepato-Somatic Index.

Key Words: Connective tissue reaction, ***Diodon hystrix*****,** ***Gymnorhynchus gigas*****, Hepato-Somatic Index, Histopathology, Plasmacyte**

Introduction

The cestodes which have been described as serious fish pathogens are almost exclusively larval forms, plerocercoids, of Pseudophyllidea and Trypanorhyncha. Despite their significant role in causing diseases in fish (Dogiel et al. 1958, Williams 1967, Sindermann 1970), information on the occurence, distribution and even the taxonomy of plerocercoids is scanty and the details regarding pathogenicity of these larvae on their hosts are lacking. The present paper deals with the nature of infection of the liver of *Diodon hystrix* by *Gymnorhynchus gigas* plerocercoid.

Materials and Methods

Host fishes, collected from shore-seines operating at Shankumughom and Valiyathurai, Trivandrum during November 1974-

April 1975 were brought to the laboratory and examined for the presence of liver infections. The total body weight, the standard length and the sex of the fish were recorded. Uninfected livers were excised entirely, blotted on filter paper and weighed. Parasites were dissected out from infected livers and the weights of the liver tissue and the parasites were also recorded. For histopathological studies, parts of both uninfected and infected liver with the parasite *in situ* were fixed in Helly's fluid. Paraffin sections cut at 7 to 10μ were stained using Azan (Heidenhain) stain.

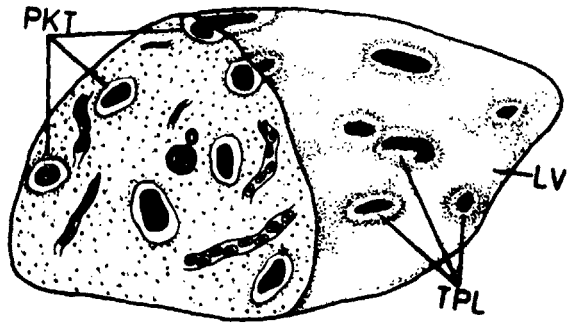

Figure 1 *Diodon hystrix* liver showing location of *Gymnorhynchus gigas* plerocercoid (Diagrammatic)

LV, liver; **PKT**, pockets containing parts of blastocyst; **TPL**, transparent patches on the liver surface

Observations

A total of 563 specimens of *D. hystrix* were examined and 9.6% were infected with *Gymnorhynchus gigas* plerocercoids with site of infection as liver. Presence of translucent patches on the surface of the liver indicated sign of infection and represented parts of the connective tissue cyst formed around the blastocyst of the plerocercoid. The anterior end of the blastocyst, containing the plerocercoid, is encysted in a folded fashion, towards the centre of the liver mass. Occasionally, however, a part of the cyst is visible externally towards the centre of the ventro-lateral aspect of the liver. In vertical sections the cut surfaces of the liver appear to have a number of circular, elliptical or irregular areas containing the blastocyst (figure 1). No apparent change in the consistency and colour of the liver is evident as a result of infection.

Invariably, each infected liver harbours only one parasite. The average length of the blastocyst is 33.3 cm (figure 2) in contrast to the average length of the liver–7.4 cm. Unless extreme care is taken it is virtually impossible to separate the blastocyst from the liver tissue without damage.

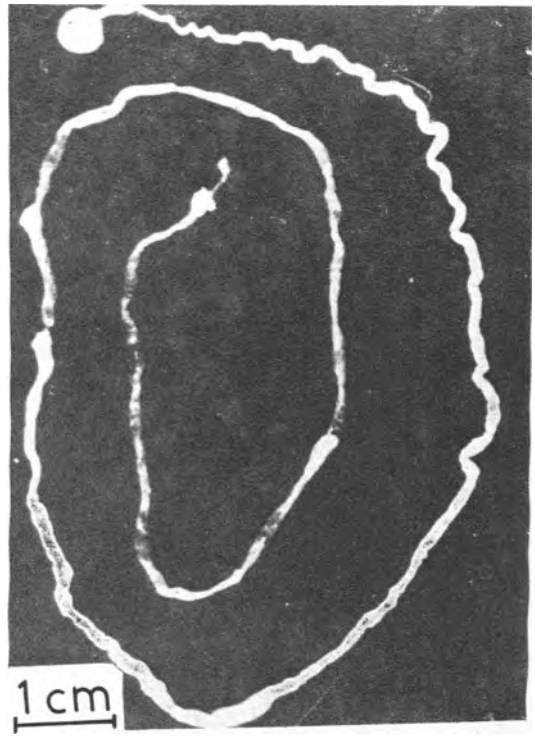

Figure 2 *Gymnorhynchus gigas* plerocercoid (blastocyst) from the liver of *Diodon hystrix*

Incidence and intensity of infection in relation to months of occurrence of the host fish, their sex and length

The incidence of *G. gigas* infection during November 1974 to April 1975 period was (9.6%) with more males (12.1%) than females (8.1%) being infected. Invariably each infected liver harboured only one plerocercoid. The percentage incidence of infection was the highest in November (27.4%), decreasing progressively in subsequent months so that by March no fish was found infected (figure 3).

Fish ranging from 11.5 to 17.5 cm in standard length alone were infected. The highest incidence of infection (11.9%) was in the 13.5–15.5 cm group and the lowest (4.4%) in the 15.5–17.5 cm group (figure 4).

Pathology of infection

Structure of the uninfected liver

The liver of *D. hystrix* is a large, spongy, yellow or pale brown mass occupying almost the entire right half of the body cavity. It is apparently triangular in shape, tapering posteriorly, when viewed from the dorso-lateral side. The ventro-lateral side, which in life is in close apposition to the rest of the visceral structures, has an anterior and a posterior depression separated by a short, tongue-shaped projection of the liver mass. The anterior depression accommodates the right ventral part of the air bladder and the posterior is so shaped as to lodge the ventro-lateral part of the right gonad.

In transverse sections the liver presents the following structure (figures 5, 6, 9).

The liver is covered over by a thin layer of connective tissue forming the capsule. As in the case of higher vertebrates, the liver of *D. hystrix* is also made up of lobules. In sections the lobules are clearly demarcated from each other by interlobular

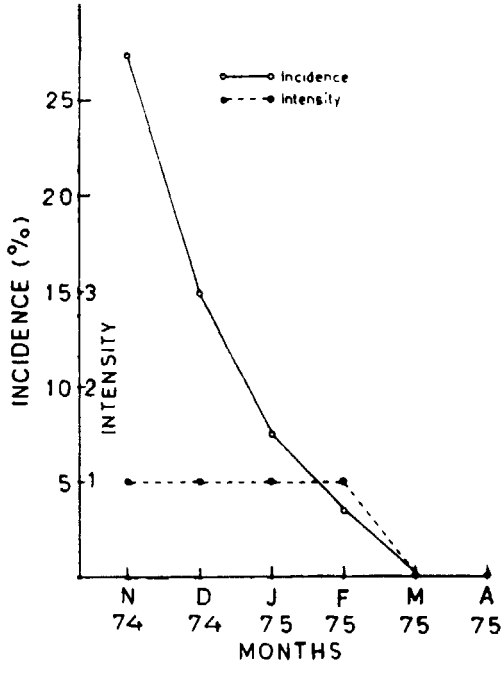

Figure 3 Incidence and intensity of *Gymnorhynchus gigas* plerocercoid infection in different months

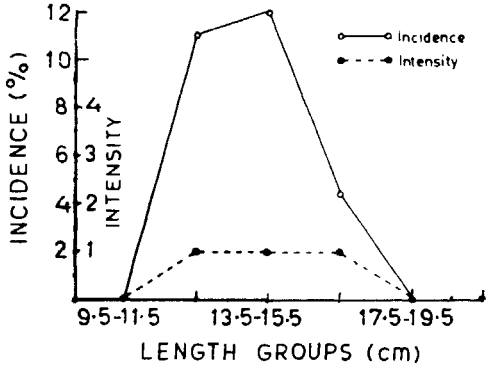

Figure 4 Incidence and intensity of *Gymnorhynchus gigas* plerocercoid infection in different length groups of the fish

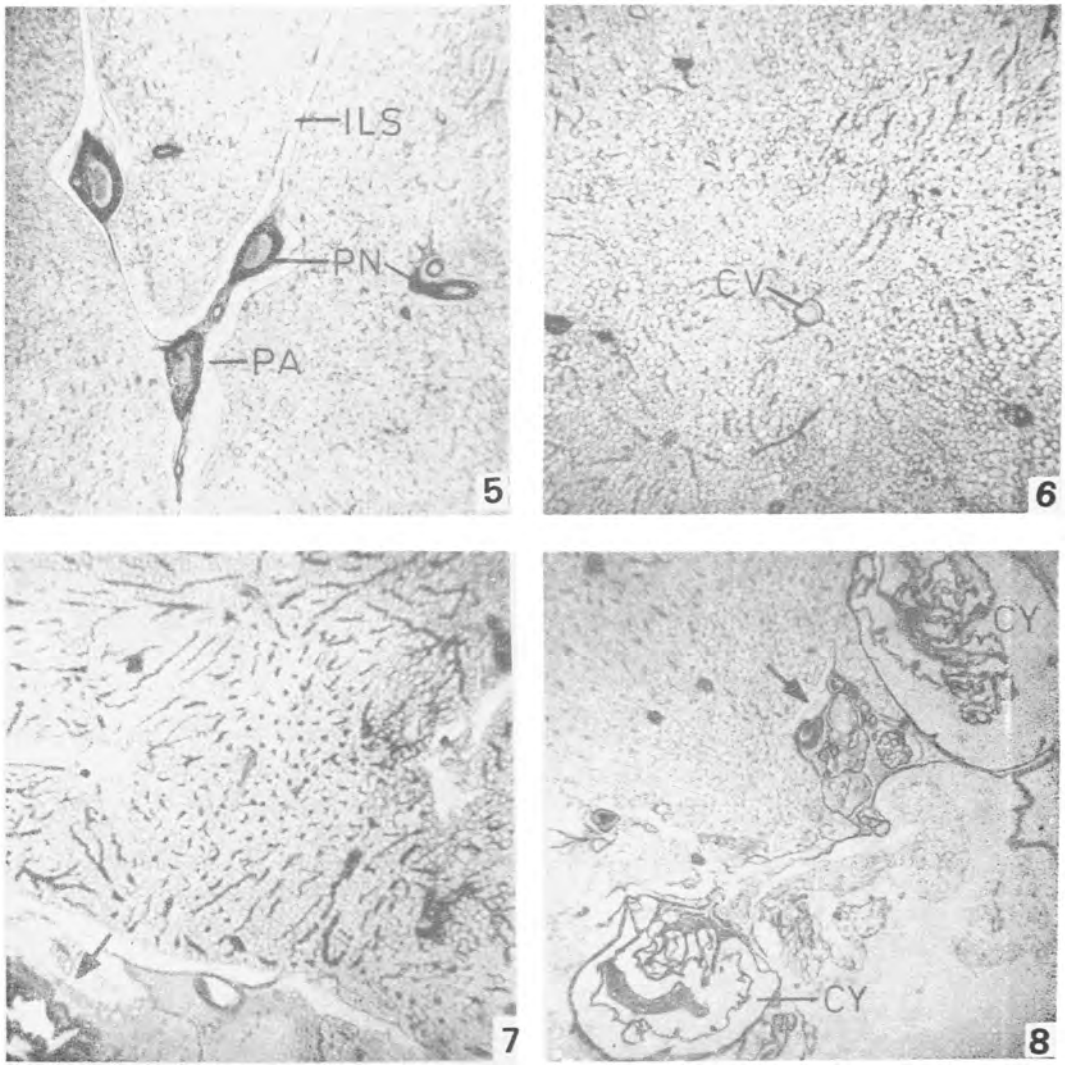

Figure 5 *Diodon hystrix*—section of uninfected liver showing interlobular space (ILS) portal area (PA) and pancreatic tissue (PN) (× 25)

Figure 6 *Diodon hystrix*—section of uninfected liver showing central vein (CV) (×25)

Figure 7 *Diodon hystrix*—section of liver infected with *Gymnorhynchus gigas* plerocercoid to show increased number of hepatic sinusoids. Arrow indicates part of the parasitic cyst. Note destructed hepatic 'parenchyma' around the cyst (×25)

Figure 8 *Diodon hystrix*—section of liver infected with *Gymnorhynchus gigas* plerocercoid showing parasitic cyst (CY) and cirrhotic lesions (arrow) (× 25)

spaces. Each angle of the apparently hexagonal liver lobule is occupied by a portal area (figure 5). The hepatic lobule, the functional unit of the liver, does not seem to be made up of laminae (which in mammals appear to radiate from a central vein located at the centre of the lobule). In fact, the entire stroma of the liver of this fish is composed of oval to round vacuoles of different sizes filled with fat and the liver parenchyma cells (and blood elements) are confined to island-like formations at the junctions of the vacuoles (figure 9).

The hepatic portal vein entering the liver breaks up into branches that are distributed through the trabeculae (the extensions of the capsule between the hapatic lobules). Small arteries and bile ductules follow the portal vein. Hepatic arteries are seen among the hepatic lobules in the trabeculae. The minute branches of the interlobular portal venules and interlobular hepatic arteries open into hepatic sinusoids, sparsely distributed in the hepatic stroma. The sinusoids converge towards the centre of the hepatic lobule to enter a central vein (figure 6). Bile canaliculi are not prominent, probably masked by the fat-filled vacuoles. Bile ductules are, however, clearly visible in transverse sections.

The hepatic arterioles are surrounded by deep staining pancreatic tissue, amidst the cells of which are seen lightly staining areas representing the islets. The pancreatic cells are loaded with small granules—ferment granules (Karandikar & Thakur 1954) or Zymogen granules.

Hepatic cells are polyhedral, confined to small islands formed at the junctions of the fat-vacuoles. Often two or three cells are found together. The hepatic cells are uninucleated. Occasionally cells having two nuclei are also observed. The nuclei are more or less round with distinct but scanty chromatin material and often with two distinct nucleoli.

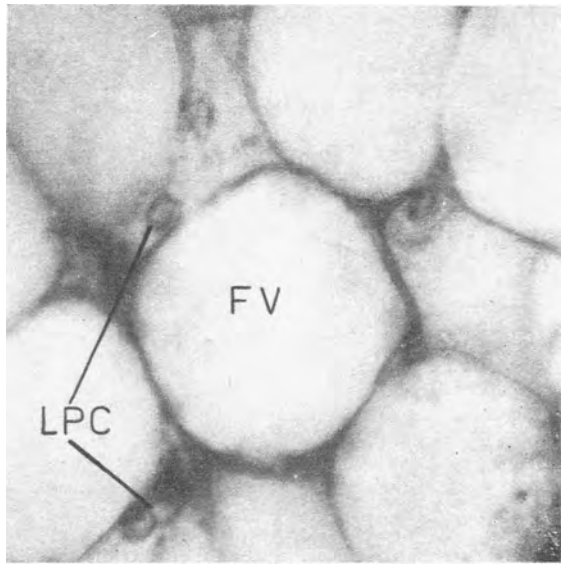

Figure 9 *Diodon hystrix*—section of uninfected liver showing fat-vacuoles (FV) and liver parenchyma-cells (LPC) (700)

Histopathology

The most conspicuous histopathological change in infected liver is the increased number of hepatic sinusoids (figure 7). Extensive connective tissue reaction around the blastocyst is evident, resulting in the encapsulation of the blastocyst (figure 8). Destruction of liver 'parenchyma' is evident around the embedded parts of the blastocyst. In some cases a sort of cirrhotic change of the tissue is also noticeable (figure 8).

Hepatic cells and fat vacuoles, except those immediately around the parasitic cyst, are quite normal in all respects. However, infiltration of a large number of inflammatory cells, especially acidophilic plasmacytes (Radhakrishnan et al. 1976) is evident throughout the infected liver.

Effect of Infection on Hepato-Somatic Index

Although fish of the 11.5–13.5, 13.5–15.5 and 15.5–17.5 cm groups only were infected, an attempt was made to study the effect of the infection on the Hepato-Somatic Index (HSI).

As is clear from the results presented in tables 1 and 2, both the total body weight and the liver weight were adversely affected by the infection, there being conspicuous decrease in both parameters.

The observed reduction in total body weight could result from the loss of liver weight; the weight of body minus the weight of liver (absolute body weight) remaining constant or much unaltered. It was, hence,

Table 1 Diodon hystrix—*Body weight of uninfected and infected male and female of different length groups*

Length group (cm)	Body weight (g)					
	Uninfected fish			Infected fish		
	Male	Female	Combined	Male	Female	Combined
9.5–11.5	92.68	91.39	92.00	—	—	—
11.5–13.5	127.07	119.93	125.07	110.54	107.83	109.53
13.5–15.5	141.04	144.21	142.63	129.57	136.05	133.25
15.5–17.5	181.08	180.82	181.00	158.70	153.60	156.15
17.5–19.5	239.20	246.35	243.13	—	—	—
19.5–21.5	368.00	327.45	350.66	—	—	—

—Data not available

Table 2 Diodon hystrix—*Liver weight of uninfected and infected male and female of different length groups*

Length group (cm)	Liver weight (g)					
	Uninfected fish			Infected fish		
	Male	Female	Combined	Male	Female	Combined
9.5–11.5	11.04	11.59	11.32	—	—	—
11.5–13.5	13.47	12.70	13.09	8.92 (1.98)	9.86 (1.84)	9.28 (1.93)
13.5–15.5	16.54	17.47	17.13	13.75 (2.89)	13.39 (2.94)	13.54 (2.92)
15.5–17.5	23.64	22.12	22.89	18.63 (3.93)	15.20 (3.92)	16.92 (3.93)
17.5–19.5	26.44	27.75	27.10	—	—	—
19.5–21.5	39.62	34.24	38.05	—	—	—

—Data not available

Numbers in parentheses denote weight of parasite

decided to study the relation of the liver weight to total body weight (Hepato-Somatic Index; HSI) which was calculated from the following formula suggested by Arme and Owen (1967).

$$HSI = \frac{liver\ weight}{total\ body\ weight} \times 100$$

From the results presented in table 3 it is evident that HSI is lower in infected fish, suggesting that the deteriorative effect of the infection on the liver weight is more severe than that on the absolute body weight.

Another significant observation in this connection is that the weight of the parasite increased with increasing length of the fish. From the data available on the infection in the three length groups of the fish, the increase in the weight of the parasite is seen to be, roughly 1.0 g per 2.0 cm increase in the length of the fish (table 2).

Discussion

As with other helminth infections in *Diodon hystrix*, *Gymnorhynchus gigas* plerocercoid infection of the liver also showed a decreasing trend from November to April. Because the life-cycle of the parasite is unknown and since the host fish are not available throughout the year along the South-west coast, positive conclusions regarding the decline in the incidence of infection in warmer months are difficult at present. It may be that by the onset of warmer season the parasites begin their development in the final host. It appears that the incidence of a serious sporozoan infection (*Glugea* sp.) of the liver, the acute stage of which reaches its peak in this fish by March, has some relationship with the cestode infection. The noted decline in *G. gigas* infection by the time the fish has been considerably weakened by the ensuing sporozoan infection may be an effective adaptive mechanism by which the host population is preserved. It is noteworthy that simultaneous infection of the liver of *D. hystrix* by the cestode and the sporozoan parasites, both of which are of serious concern to the host, has not hither to been encountered. This also may be an adaptive mechanism by which the host population keeps the effect of parasitic infections at the minimum compatible level for its continued existence. However, the actual mechanisms involved remain obscure.

Table 3 Diodon hystrix—*Hepato-Somatic Index (HSI) of uninfected and infected male and female of different length groups*

Length group (cm)	Hepato-Somatic Index					
	Uninfected fish			Infected fish		
	Male	Female	Combined	Male	Female	Combined
9.5–11.5	12.02	12.86	12.51	—	—	—
11.5–13.5	11.11	10.63	10.83	8.09	9.04	8.44
13.5–15.5	11.80	12.32	12.15	10.61	9.84	10.16
15.5–17.5	13.25	12.15	12.61	11.74	9.90	10·83
17.5–19.5	11.11	11.27	11.20	—	—	—
19.5–21.5	10.77	10.47	10.50	—	—	—

—Data not available

In fact, the histopathological changes as a result of *G. gigas* infection of the liver are not very serious. Nevertheless, the infection adversely affects the condition of the fish by lowering both the body and liver weights. The observations on the haematological and biochemical changes in infected fish (Radhakrishnan & Nair, Unpublished) show that *G. gigas* infection adversely affects the liver function.

Acknowledgements

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