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Keywords. *Caryophylliasis*; *Clarias batrachus*; *Lytocestus indicus*; *Djombangia penetrans*; histopathology.

1. Introduction

Cestodes, caryophyllids in particular, are well known to produce certain adverse effects on their piscine hosts (Amlacher 1961; Musselius *et al* 1963; Akhmetova 1966; Mackiewicz *et al* 1972; Bauer *et al* 1973). These include mechanical blockage of the gut lumen (Scherban 1965), production of lesions (Mackiewicz *et al* 1972; Hayunga 1979a, b) and in the physiological state of the host thereby predisposing it to other infections (Jara and Szerow 1981a, b; Kadav and Agarwal 1982, 1983a, b).

In the Indian subcontinent though the dominant hosts for caryophyllids are the siluriform and cypriniform fishes, *Clarias batrachus* (L.) and *Heteropneustes fossilis* (Bloch) are the chief hosts (Mackiewicz 1981). During our studies on the helminth parasites of fishes in north-east India, *C. batrachus* was found to be frequently and mainly parasitized by the caryophyllids, *Djombangia penetrans* Bovien 1926; *Lytocestus indicus* (Moghe 1925) Woodland 1926 and to a lesser extent by other species of *Lytocestus* such as *L. filiformis* Fuhrmann and Baer 1925, *L. birmanicus* Lynsdale 1956 and *L. longicollis* Rama Devi 1973 which occurred concurrently with the dominant species, and formed multiple infection.

In the present study, histopathological changes in the intestinal wall of the host subsequent to infections by these parasites in single or multiple infection are reported.

2. Materials and methods

The intestinal tissue, with parasite *in situ*, was fixed in Bouin's fixative, and processed for routine histological investigations. Paraffin sections of 6–7 μm thickness were stained following Mallory-Heidenhein's triple staining technique. Sections of intestinal tissue from uninfected host fish served as controls.

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3. Results

The normal intestinal wall (figure 1) of *C. batrachus* is composed of mucosa (columber epithelium consisting of absorptive and mucus secreting cells), submucosa (highly vascularized and extending into the villi as lamina propria), muscularis (comprising inner circular and outer longitudinal muscle fibres) and the outermost coat, serosa, with a rich vascular supply (Khanna 1980).

With *D. penetrans*, *L. indicus* and caryophyllidean-mix infections the intestinal wall revealed different pathological changes.

3.1 *L. indicus*

This species penetrates deep into the muscularis layer (figure 2). It was observed that despite this there was no nodule formation. At the site of scolex attachment to the intestinal wall mechanical displacement and compression of tissue layers such as mucosa, submucosa and muscularis were noticed. Due to excess pressure exerted by the scolex, in severe cases the submucosa became hyperplastic (figure 3). No leukocytic infiltration was observed; however, in some cases a thin mucoid interface between the host-tissue and the scolex was observed. Loosening of muscle fibres was also evident. Apart from these changes, infection with 8–10 worms per sq. cm. area seemed to cause some overcrowding effect, resulting in the blockage of the intestinal lumen.

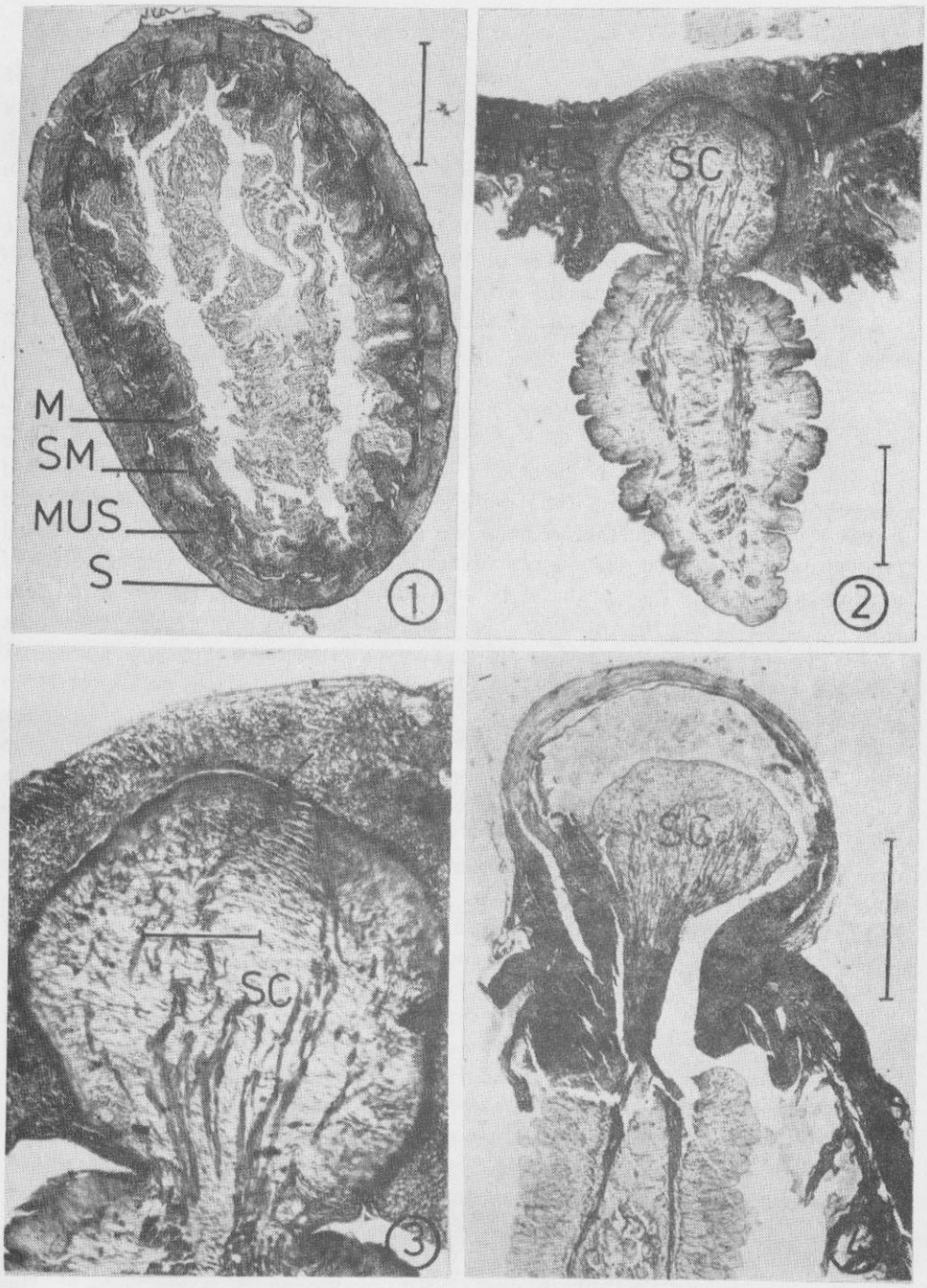
3.2 *D. penetrans*

A sucker at the tip of the worm aids in its firm attachment to the host's tissue. Frequently nodular swellings were observed on the outer wall of the intestine, caused by the penetration of the parasite into the serosa. Formation of nodules led to the production of tunnels through the intestinal wall in which the neck portion of the worm remained embedded. While the scolex was encapsuled in the lumen of the nodule, the body remained free in the lumen of the intestine. As a result of compression of the mucosal folds, the submucosa and the muscularis formed plaques at the site of attachment. The compressed tissue layers appeared thickened on the two sides of the tunnel. However, the nodular lining did not show such thickening. Each nodule contained only one worm, but debris and necrotic tissue were seen near the scolex. Besides, an interface layer separated the host's tissue from the holdfast of the worm (figure 4).

Like *L. indicus*, *D. penetrans* also caused mechanical blockage of the intestinal lumen.

3.3 *Multiple infections of caryophyllids*

Generally, multiple infection comprised as many as 3 to 4 different species and a total of 10–12 worms concurrently parasitised a single host. Most of these worms had elongated bodies and hence occupied a considerable niche length resulting in mechanical obstruction of the gut. The pathogenicity was not severe because no ulceration or nodular formation took place in the host's intestinal wall, but the intestinal villi or the mucosal folds got compressed.



Figures 1–4. Histopathology of the host tissue. **1.** Normal intestinal tissue of *C. batrachus* showing the different layers. **2.** *L. indicus* anchored to the intestine, illustrating penetration up to the muscularis layer (scale bar = 0·5 mm). **3.** The same at higher resolution showing hyperplasia of the muscularis and a thin interface layer (arrow) (scale bar = 0·15 mm). **4.** Section through a nodule harbouring the scolex of *D. penetrans*. Note the muscularis thickening adjacent to the tunnel (arrow) (scale bar = 0·5 mm).
(M, Mucosa; SM, submucosa; MUS, muscularis; S, serosa; Sc, scolex).

4. Discussion

The distribution of *L. indicus*, *D. penetrans* and species constituting the multiple infection within the host's intestine shows a definite habitat preference depending upon the type of scolex. While *L. indicus* and *D. penetrans* inhabited the duodenum, species such as *L. birmanicus*, *L. longicollis* and *L. filiformis* were always found in the anterior and posterior loops of the intestine.

The effects on the host seem to be related to the mode of attachment of the scolex (Mackiewicz *et al* 1972; Bauer *et al* 1973). Thus, deep penetrating scoleces of *L. indicus* and *D. penetrans* caused serious damage to the host's intestinal wall.

L. indicus produced shallow ulcers within the host's tissue causing destruction of villi, mucosa and submucosa. These observations are in conformity with those of Satpute and Agarwal (1974), Ahmed and Sanaullah (1979), Bose and Sinha (1981) and Niyogi and Agarwal (cited from Agarwal 1985). However, *L. indicus* was never found to reach the serosa and no leukocytic infiltration was observed. Further, the pinocytotic vesicles in the holdfast end of the worm as observed by Niyogi and Agarwal (cited from Agarwal 1985) were also found lacking. Since no degenerated tissues were seen within the folds of the scolex end, these (folds) could not be considered pinocytotic vesicles. The pronounced tissue reaction expressed as hyperplasticity of the submucosal layer may probably be attributed to the secretion of the gland cells that are present in the holdfast end (unpublished results). Such gland cells (='Frontaldrüsen' and 'Faserzellenstränge') are reported to be present in the scolex and neck region of many caryophyllids (Janizewska 1954; Richards and Arme 1981) and assist in attachment (Hayunga 1979a) or penetration of the host's tissue by secreting lytic enzymes (Slais 1961). The highly developed musculature in the holdfast end may also be responsible for exertion of pressure in the tissue layers leading to their compression. However, the mechanical obstruction is caused due to the occurrence of parasites in clusters.

The pathogenicity caused by *D. penetrans* can be compared to that by other caryophyllids viz., *Hunterella nodulosa*, *Biacetabulum biloculoides*, *Monobothrium ulmeri* and *M. ingens* which also produce pronounced host reaction in the form of nodules (Mackiewicz *et al* 1972). Lacking a specialized holdfast, *H. nodulosa*, like *D. penetrans*, produces a large nodular thickening with a pronounced active chronic inflamatory reaction. However, the nodular lining of *D. penetrans* appears to be necrotic with debris in the pit and the nodule is single chambered, harbouring only one worm unlike the nodules of *H. nodulosa* which some times have more than one chamber containing as many as 127 worms up to various stages of development (Mackiewicz and McCrae 1962). Debris and necrotic tissue surrounding the scolex are also found in the nodules formed by *B. biloculoides*, *M. ingens* and *M. ulmeri*. However, the size of the nodule produced by *D. penetrans* appears to be the smallest. Histologically, the nodules of *D. penetrans* appear similar to those produced by *Polyonchobothrium clarias*, a pseudophyllidean in the gall bladder of *Clarias mossambicus* (Wabuke-Bunoti 1980), in having 3 layers namely, the continuous outer epithelial layer, a granulomatous sub-epithelial layer and the innermost fibrous connective tissue layer; however the last-mentioned two layers are not highly vascularized.

While Mackiewicz *et al* (1972) associated the changes with proteolytic secretions of the frontal glands, Hayunga's (1979b) observations on *H. nodulosa* revealed no evidence of proteolysis or necrosis of host tissue. In his opinion, probably the eosinophilic matrix was primarily an adhesive layer which acted as a strong irritant, and the latter combined with a strong contraction of the worm caused ulceration and loss of epithelium. However, the occurrence of necrotic debris in the present study is suggestive of the presence of proteolytic enzymes in the holdfast end. This needs further investigation.

Another remarkable difference between the host reaction produced by *D. penetrans* and that by other nodule-forming caryophyllids is that in none of the latter does there occur a muscular thickening on the sides of the penetration site. As a result of penetration or burrowing deep up to the serosa by *D. penetrans* the tissue layers, across which it penetrates, get thickened. Similar observations were made by Satpute and Agarwal (1974) and Ahmed and Sanaullah (1979) on *Djombangia* spp. infections in *C. batrachus*. The nodule formation which seems to be an inflamatory response of the host provides a sheltered habitat and firm anchorage to the worm. This host response is an example of exploitation by the parasites of its host defence mechanisms to its own advantage (Hayunga 1979a).

The pathological changes manifested in the form of shortening of intestinal villi or compression of the mucosal folds due to multiple infection are probably a consequence of pressure necrosis. Except the alterations in the absorptional area of the intestine, and mechanical blockage of its luminal space, no serious host reaction is produced due to multiple infection. Multiple infections of caryophyllids are common (Mackiewicz *et al* 1972) and seemingly have no detrimental effect on the host (Agarwal 1985).

The present study also suggests that the mode of attachment is primarily responsible for triggering significant host reaction. While single worm of *L. indicus* or *D. penetrans* results in shallow ulcers or nodule formation, respectively, multiple infection results only in the atrophy of the intestinal villi, and the host reaction elicited is minimum, probably because the mode of attachment of the worms is very weak.

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