

ON DIPLOSTOMOSIS OF THE GRASSCARP FRY

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The diplostomosis of various fish species is well known and the life cycles of several *Diplostomum* species have been investigated in great detail, especially by SHIGIN (1964, 1968, 1969). The developmental cycle of *Diplostomum spathaceum* (Rud., 1819), most frequently occurring among fishes in Hungary, was followed up by CICHOWLAS (1961).

The pathogenic effect of *Diplostomum* parasites has been little studied. Several authors reported that the establishment of metacercariae in the lens caused blindness. Growth retardation by diplostomosis was observed by USPENSKAYA (1957) in the brown trout and by MINDEL (1963) in *Coregonus* sp. MINDEL also observed losses and so did EGELSKIY (1965) among infected sturgeon fry, which had just started to feed. In both cases, migrating larvae were responsible for the losses.

The development of the larval stages of *Diplostomum* species was studied by ERASMUS (1958, 1959) on fishes experimentally infected with *Cercaria-X* species from different *Lymnaeidae*; he investigated the mode of infection, cercaria migration within the host and the relationship between the counts of infective cercariae and losses. As ERASMUS did not precisely determine the species of the metacercariae establishing themselves in the lens, it can only be tentatively inferred that he was working with *Diplostomum spathaceum*.

Diplostomum-infestation has also been known among grasscarps (*Ctenopharyngodon idella*), silver carps (*Hypophthalmichthys molitrix*) and marmor carps (*Hypophthalmichthys nobilis*) (AKHMEROV, 1963; SZAKOLCZAI and MOLNÁR, 1966; BAUER *et al.*, 1969; SHIGIN, 1971), but deaths from this parasitosis have not yet been observed. The investigations were focused on cataractic lesions of the lens in advanced diplostomosis. SHIGIN (1971) demonstrated that diplostomosis causes growth retardation also in grasscarp and AKHMEROV (1963) pointed out that *D. spathaceum*-metacercariae are pathogenic for herbivorous fishes, but presented no evidence.

In this paper an outbreak of diplostomosis which caused great losses among the grasscarp fry is reported. The circumstances of death, symptoms, gross lesions and a histological follow-up of larval migration are described in detail.

Material and Methods

The examinations were conducted on 1.7-2.5 cm long, 3-week-old grasscarp fry procured from a pond farm. The young fishes were sectioned under a stereo-microscope and impression smears from different organs were investigated by light microscopy.

For histological examination, the fishes were fixed *in toto* in 4% formalin solution. The sections were cut longitudinally, so as to comprise the eye and were stained with haematoxylin and eosin or Farkas-Mallory's technique.

Species determinations were carried out according to SHIGIN (1968) on metacercariae collected from fingerlings died during the outbreak and later from survivors. In the first case, care was taken to use older stages, already showing the species characteristics.

Results

In July 1972, an outbreak causing high losses occurred among the 3-week-old, 1.7–2.5 cm long grasscarp fry in a pond farm of Eastern Hungary. The fingerling pond in which the outbreak was observed, had been stocked with equal populations of newly-hatched grasscarp and silver carp fry some time ago.

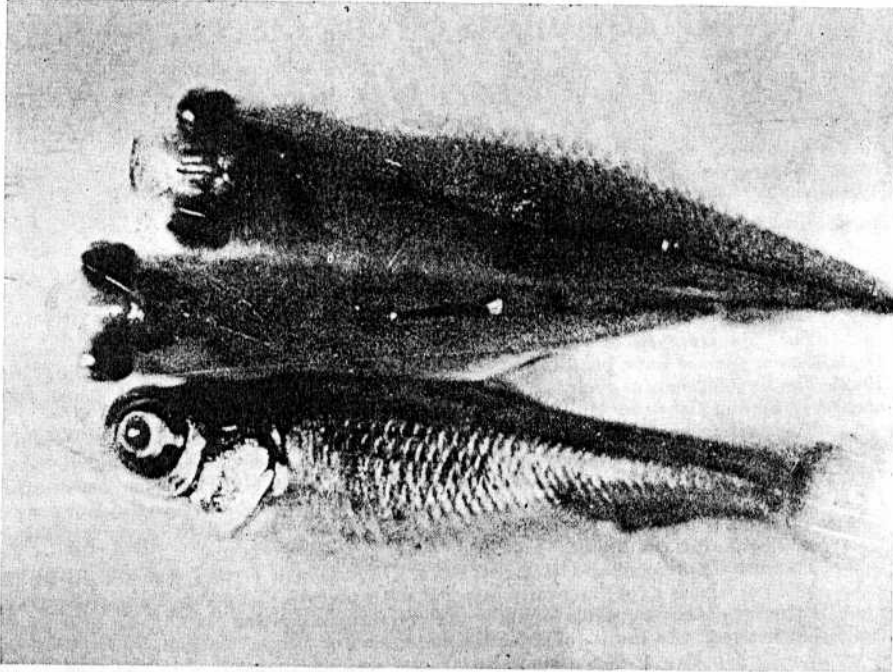


Fig. 1. Young 2.5 cm long grasscarp diseased in diplostomosis. Note protruding eyes and haemorrhages in the orbital cavity and skull area

Within a short time, 90% of the young grasscarps died of the disease, but no losses occurred among the silver carps, which were of the same age and size. The grasscarps taken from the pond for laboratory examination died in our aquaries within 48 hours, whereas all silver carps collected along with them survived.

In pond and aquary alike, the diseased fry showed characteristic symptoms. They did not respond to external stimuli and could easily be caught.

Some of them performed compulsive movements, usually in a rotating manner and the still weaker individuals floated on the water surface with the abdominal side upwards. The vivid red colour and congested blood vessels of the skull region were well-visible at first sight. The swollen eyeballs were nearly double of the normal size, protruded from the orbital cavity and were vivid red like the top of the skull (Fig. 1). Some specimens had already lost one eye. The opaque, often milky white lens was scarcely visible because of haemorrhages in the ocular chamber.

Parasitological section

Many *Diplostomum* larvae, in different stages of development, were found in the lens. Migrating *Diplostomum* cercariae occurred in the vitreous body and other parts of the eye as well as in the brain, heart and muscles. The migrating stages were smaller than those which established themselves in the lens and, unlike metacercariae, they had a well-visible gut and ventral sucker; the suckers and integument were covered by spines. Most larval stages (20—25 per host) localized in the lens, but relatively many (4—5 per host) were present elsewhere in the eye. The average larval number in other body regions was 7—10 migrating stages per host. Except for some *Trichodina* on the gills, no other parasite species was found.

The silver carps were infested by *Diplostomum* to a similar degree as grasscarps and they also had coccidiosis, but neither symptoms nor losses occurred among them. Grasscarps from four adjacent fingerling ponds of the same farm were examined as controls; these had a much lower degree of metacercarial infestation, harbouring on the average 5—15 parasites per host. Certain severely infested individuals of the control lot showed, nevertheless, the same symptoms as described above.

Examination on metacercariae

Among the metacercariae collected from the infected fry and other grasscarps all stages which already had shed the larval integument showed the features described by SHIGIN (1968) as characteristic of *Diplostomum spathaceum* (Rud., 1819).

Histological examinations

The metacercariae which established themselves in the lens, above all at its periphery (Fig. 2), were easily demonstrable histologically. They caused slight lesions: an epithelial proliferation was only seen at the site of larval penetration. Cercariae migrating towards the lens were found in different parts

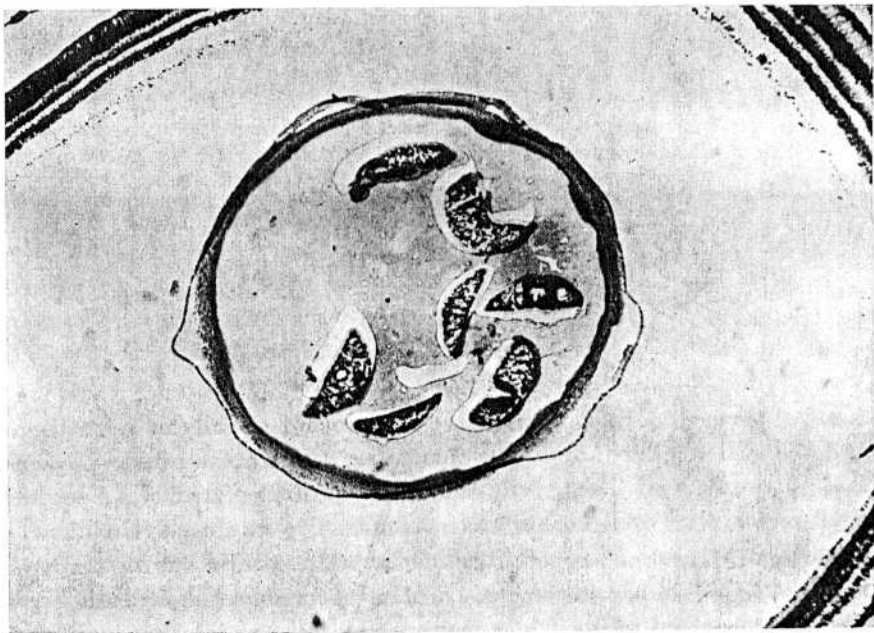


Fig. 2. Young metacercariae established in the lens. Haematoxylin and eosin staining, $\times 200$

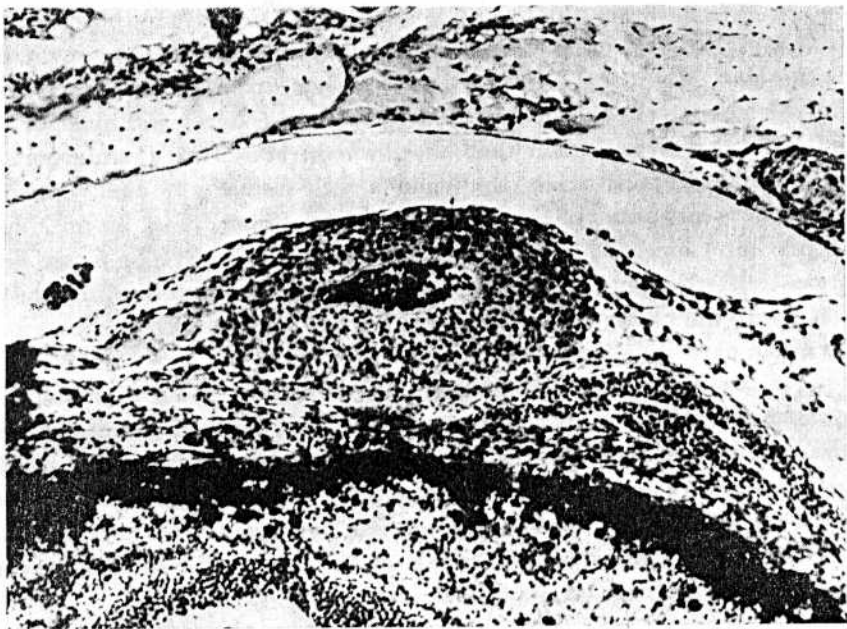


Fig. 3. *Diplostomum-cercaria* surrounded by proliferative tissue close to the pigmented chorioidea

of the eye, in the cornea, retina and vitreous body, surrounded by cells characteristic of the localization and by blood corpuscles. In certain cases, however, the cercariae were surrounded by granulation tissue (Fig. 3), occurring above all in the orbital cavity, but also in the periocular connective tissue and inside

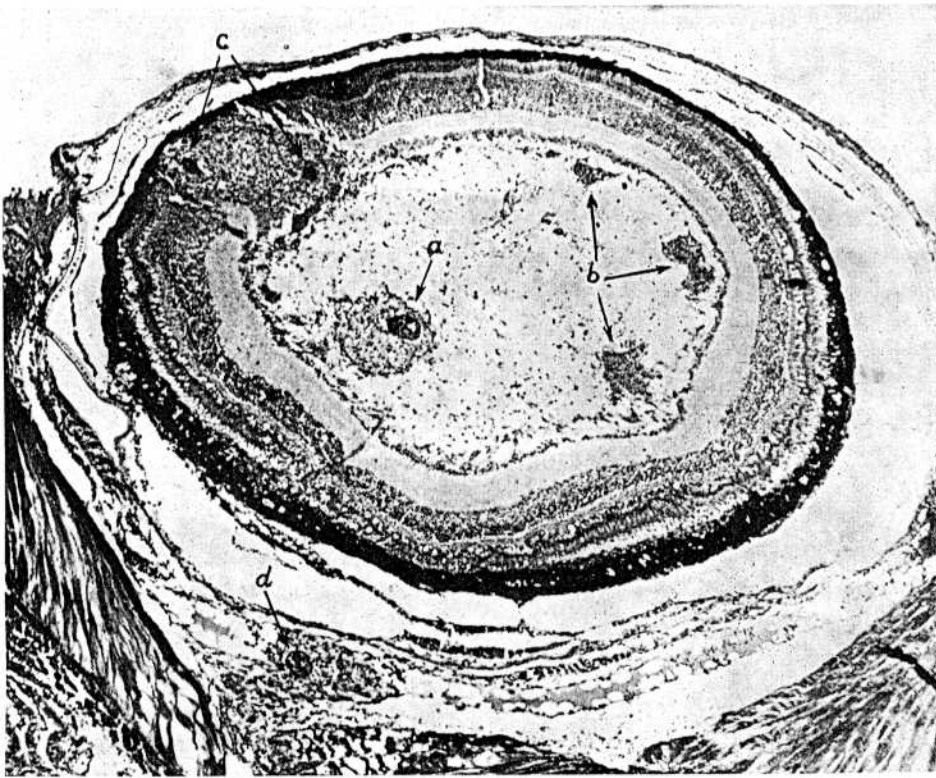


Fig. 4. Tissue reaction around a cercaria established in the ocular cavity of the grass carp. *a*, Cercaria in the vitreous body; *b*, haemorrhages in the vitreous body; *c*, transfixed cercaria in the retina; *d*, cercaria surrounded by proliferative tissue in the choroid. Haematoxylin and eosin stain, \times about 60

the eyeball. Granulation was most frequently seen around the larvae localizing in the chorioid body, but it also occurred in the retina and vitreous body (Fig. 4). No such tissue reaction was seen around larvae migrating in other body regions, e.g. in the heart (Fig. 5) and skull area (Fig. 6). In addition to tissue reactions, there were extensive haemorrhages in the eye chamber, vitreous body, chorioid and cerebral meninx (Fig. 7).



Fig. 5. Heart of grass carp in longitudinal section. Note the cross-section of cercaria between auricle and ventricle

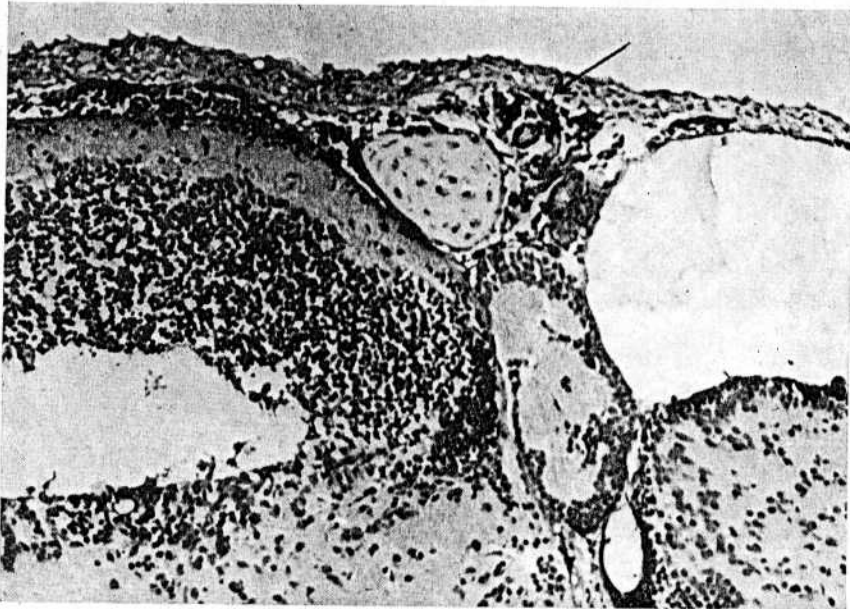


Fig. 6. Skull area of grass carp. Note the penetrating cercaria under the epithelium

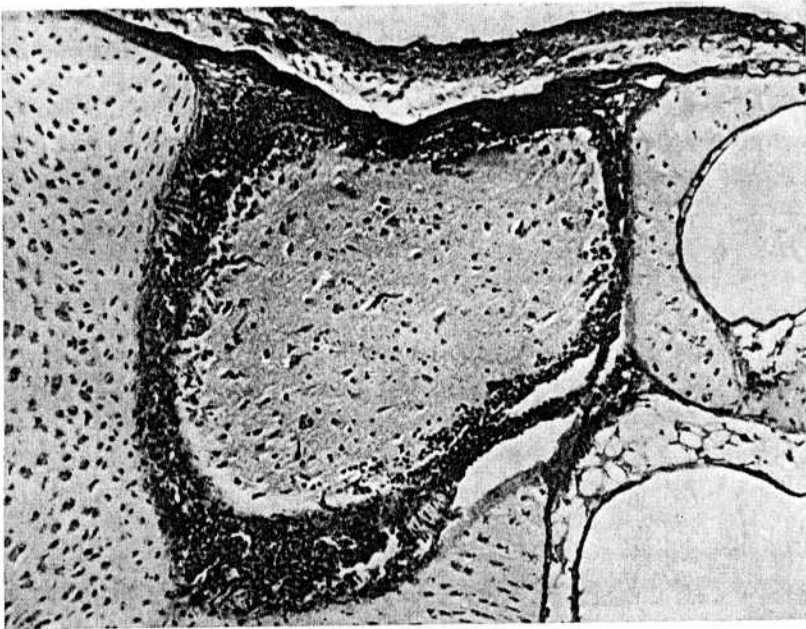


Fig. 7. Haemorrhages in the meninx of a grass carp

Discussion

It was reported earlier (SZAKOLCZAI and MOLNÁR, 1966) that in Hungary, the herbivorous fishes were much more extensively infested with *Diplostomum spathaceum* metacercariae than the common carp and that 5–8 cm long, one-summer grasscarps were not infrequently found to harbour 200–300 metacercariae in the ocular lens. The damages of metacercaria-diplostomosis could not be estimated because *Diplostomum* infestation is so frequent among grasscarps that no appropriate controls were available. Thus we were unable to clarify the relationship of the growth retardation described by USPENSKAYA (1957), MINDEL (1963) and SHIGIN (1971) with the diplostomosis-associated lenticular cataract, because this was present in practically all grasscarps.

As in the outbreak studied, other parasitoses and a dietary failure could be excluded, it seems certain that it was due to a cercaria-diplostomosis, viz., the losses were caused by migrating *Diplostomum spathaceum* larvae. The fatal outcome depended on the number of larvae establishing themselves in, and migrating in, the body of the host: 20–25 sessile and 10–15 migrating diplostomulum stages killed the 1.7–2.5 cm long young grasscarps.

The lethal changes already develop during migration and the larvae establishing themselves in the lens do not seem to play a decisive role. This is in good accordance with the observation that in a similar outbreak among

common carps in the previous year the hosts did not show eye symptoms and harboured only 1—2 parasites, if any, in the lens, but migrating *Diplostomum* larvae were always present in their body. In hosts showing compulsive movements, the migrating stages were chiefly found in the brain and spinal cord.

In the outbreak studied, the grasscarps showed the characteristic symptoms of cercaria-diplostomosis, corresponding essentially with those described by PETRUSHEVSKIY and BAUER (1953).

We do not agree with MUSSELIUS (1967) who established that the grasscarp is less susceptible to *Diplostomum* infestation than the silver or marmor carps. The apparent contradiction can probably be ascribed to the dissimilar water levels of fingerling ponds in the USSR and Hungary. In this country, they are generally shallow, not allowing for the separation of the biotopes of grasscarp, silver carp and marmor carps, whence the chances of cercaria-infestation are equal for all the three species. My own experience is that when grasscarp and silver carp fry of the same age and size were reared together in one and the same fingerling pond, the former proved to be more susceptible to migrating larvae.

The severe outbreak of diplostomosis which involved the entire grasscarp population of the fingerling pond can be regarded as a warning that cercaria-infestation may be more frequent than inferred from reported cases. Unexplicable "disappearances" of fry from the pond may not infrequently have been due to this disease.

The results of histological examinations correspond with the findings of ERASMUS (1959) and suggest that cercariae can penetrate the body of the host at several sites. The occurrence of migrating cercariae in different tissues of the body also supports ERASMUS's opinion that the blood stream plays little role in larval migration towards the eye.

The route of the cercariae is marked by extensive haemorrhages, above all in the richly vascularized areas, such as the cerebral meninges and orbital cavity. Death occurs when the cercariae, being relatively large compared to the dimensions of the host, injure vitally important organs by causing haemorrhage or otherwise. In this respect the meningeal haemorrhages are more deleterious than even the severest ocular lesions.

A fatal outcome of diplostomosis seems to occur when a very young host which had not previously been infected is heavily invaded by cercariae.

The spawning of herbivorous fishes requires a relatively high temperature and thus takes place late in the season. Consequently, the rearing of the fry coincides with the appearance of the factors mediating parasite invasion, viz., snails and young birds. Exposure to *Diplostomum* infestation is therefore greater in the case of herbivorous fishes than for other cultured fishes.

It was shown histologically that part of the penetrating parasites elicit a special reaction, characterized by tissue proliferation. Proliferative tissue

was usually found within the eyeball or in its immediate surroundings. It is remarkable that the lens, the site of preference, showed only slight lesions: proliferation of the lenticular epithelium was only seen at the sites of penetration, as already observed by MAWDESLEY-THOMAS (1972).

The direct causes of tissue proliferation are not yet fully understood. It might represent the protective mechanism of the host or, as suggested by ERASMUS (1959), simply the encapsulation of cercariae having lost viability during migration. Thirdly, it may be a tissular reaction to the direct mechanical effect of the established stages or to the chemical effect of their metabolic and other products.

Summary

Infestation by cercariae of *Diplostomum spathaceum* killed 90% of the 3-week-old, 1.7–2.5 cm long grasscarp (*Ctenopharyngodon idella*) fry reared in a fingerling pond. The severely diseased hosts harboured on the average 20–25 larvae in the ocular lens and 10–15 migrating stages in other parts of the body.

Cercarial migration caused haemorrhages, visible even externally.

Most cercariae which established themselves in the eye or the adjacent areas were surrounded by proliferative tissue.

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