Journal of Fish Diseases 1978, **1**, 151–156

Intestinal lesions in grasscarp *Ctenopharyngodon idella* (Valenciennes) infected with *Balantidium ctenopharyngodonis* Chen.

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Abstract. The protozoan *Balantidium ctenopharyngodonis* is a common intestinal commensal of two-summer and older grasscarp, but it may occasionally become pathogenic under the influence of certain predisposing factors. Outbreaks of enteritis complicated by excess multiplication of balantidia occurred among the two-summer and three-summer grasscarp stock in two pond farms during the autumn and winter. Fishes in the terminal stage of disease showed hyperaemia and inflammation of the mucosa over the entire gut. In the posterior 10–12 cm of the gut a dense creamy exudate coated the mucosa. Large numbers of balantidia were found in the exudate and between the mucosal folds there were excoriations, the size of a pin-head, obviously caused by the parasites. Histological examination revealed a loss of superficial epithelium and ulcerations in the grooves between folds.

Introduction

Species of *Balantidium* are common intestinal parasites of both vertebrate and invertebrate animals. Fish balantidia were described originally from hosts indigenous in far-eastern rivers (Chen 1955; Ha Ky 1969), but recently *Balantidium ctenopharyngodonis* Chen, 1955 has been introduced into European habitats as a result of grasscarp *Ctenopharyngodon idella* (Valenciennes) importation (Musselius & Strelkov 1968; Molnár 1971). Since no precise information was available on the pathogenicity of *B. ctenopharyngodonis* grasscarp naturally infected by the parasite were studied in this laboratory for gross and microscopic lesions.

Materials and methods

The investigations were commenced in December, 1975, when balantidiosis was suspected as the cause of mass losses occurring in cultured grasscarp populations.

In a pond farm, a considerable part of the two-summer and three-summer grasscarp stock died of the condition. At the time of the examination many fish were

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0140-7775/78/0400-0151 $02.00

obviously diseased, but some apparently healthy individuals were also found. Five two-summer fish and four three-summer fish, all moribund, were taken for pathological and histological examination.

The diseased fish either swam listlessly near the water surface, or floated upside down. At autopsy, the gut was found to be empty, and the mucosa of the foregut was dark red and oedematous, with injected vessels. The lumen was filled by a jelly-like, yellowish-red, turbid mucus. In the posterior 10–12 cm of the gut, the spaces between the mucosal folds were coated by a creamy exudate, which could be easily wiped off. Beneath the exudate the mucosa was dark red and swollen in places and the vessels traversing it were injected. In the inflamed part of the gut the edges of the transverse mucosal folds were also reddened. At the base of the folds there were excoriations the size of a pinhead. Only the three-summer grasscarp were examined bacteriologically. No isolates were made from the blood and viscera, but a pure culture of *Aeromonas punctata* was cultured on blood agar plates inoculated with intestinal scrapings.

Ten two-summer fishes not showing clinical signs of disease also exhibited reddening and hyperaemia of the intestinal mucosa, but a creamy exudate was not found in these instances.

In January 1977 a similar condition occurred in another pond farm. Thirty, two-summer fishes, of which only two had shown severe signs of disease were examined. The two severely diseased fish showed enteritis, excoriations, and a creamy exudate, in the hindgut, as described above. During the period between the two outbreaks, the prevalence of balantidia was assessed in apparently healthy hosts from the latter farm, by examination of 150, 44 and 17 one-, two- and three-summer or older, grasscarp, respectively.

Samples of intestinal contents, intestinal mucus as well as mucosal scrapings were taken from several parts of the gut. The samples were examined microscopically at × 100 magnification. With one- and two-summer hosts the examinations were limited to three sampling areas, i.e. foregut, midgut and hindgut, but with older hosts, the hindgut was subdivided into three additional parts for sampling.

The intestines of two- and three-summer grasscarp were processed for histological examination. The specimens were immediately fixed in formalin, and paraffin sections were stained with haematoxylin and eosin.

Results

None of the 150 one-summer fish examined for *B. ctenopharyngodonis* was found to be infected, and among the 44 two-summer grasscarps only two larger individuals, examined in autumn harboured balantidia in the intestine. Among the three-summer and older grasscarp seven fish, examined during the period of active feeding, harboured balantidia, but among the remaining 10, studied late in autumn and winter after the feeding period, only 6 were found to be infected by the parasite.

In those pond populations in which the outbreaks of disease had occurred, all fish

examined harboured balantidia in numbers directly related to the degree of enteritis.

Many balantidia were found in the creamy exudate coating the hindgut of fish in the terminal stage of disease, and single parasites were encountered in mucosal scrapings obtained from the posterior portion of the midgut.

Apparently healthy grasscarp, taken from the same pond as the severely diseased ones, harboured relatively fewer, but still numerous balantidia.

Examination of the different intestinal segments revealed that the balantidia usually established themselves in the anterior and central parts of the hindgut.

In very heavy infections parasites also appeared in the posterior midgut, but never in the foregut.

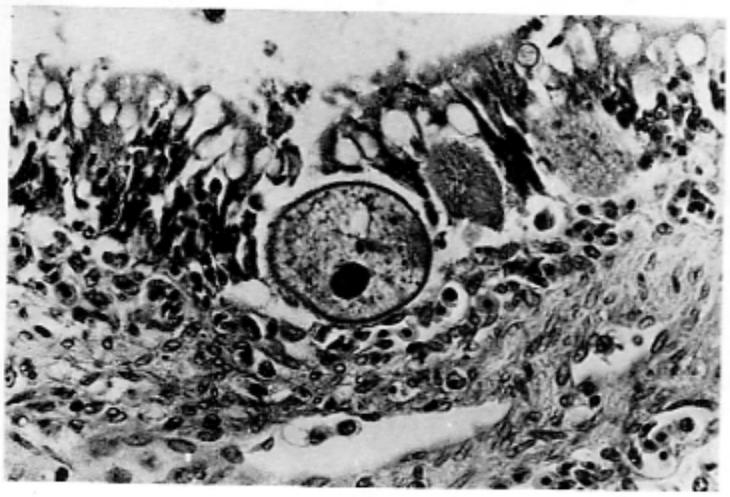

Figure 4. A balantidium established beneath the damaged epithelium. Note the dilated capillary vessels, packed with red blood cells, near the parasite and at right (H & E. ×500).

The histological sections showed the presence of many parasites in the lumen of the hindgut and also between the intestinal mucosal folds (Fig. 1). The epithelial lining was discontinuous in places between the folds (Fig. 2), and the parasites filling the spaces between folds were attached directly to the propria (Fig. 3). Some balantidia had even penetrated beneath the damaged epithelium (Fig. 4), while others had caused local atrophy of the epithelial layer (Fig. 5). Areas of serum and round cell infiltration were found in the loose fibre network of the intestinal submucosa. The inflammatory reaction was especially pronounced adjacent to the muscle layer. The capillaries of the propria were markedly dilated, and those traversing the folds were heavily congested with blood (Fig. 4). The changes occurring in the submucosa and propria were also encountered in the parasite-free anterior portions of the gut.

Histological examination showed that mildly infected hosts also harboured balantidia in the intestine, but in such cases the parasites were invariably localized in the lumen, along the intact epithelial lining. Submucosal round cell infiltration and vasodilatation were seen in certain parts of the gut, chiefly near the anal opening,

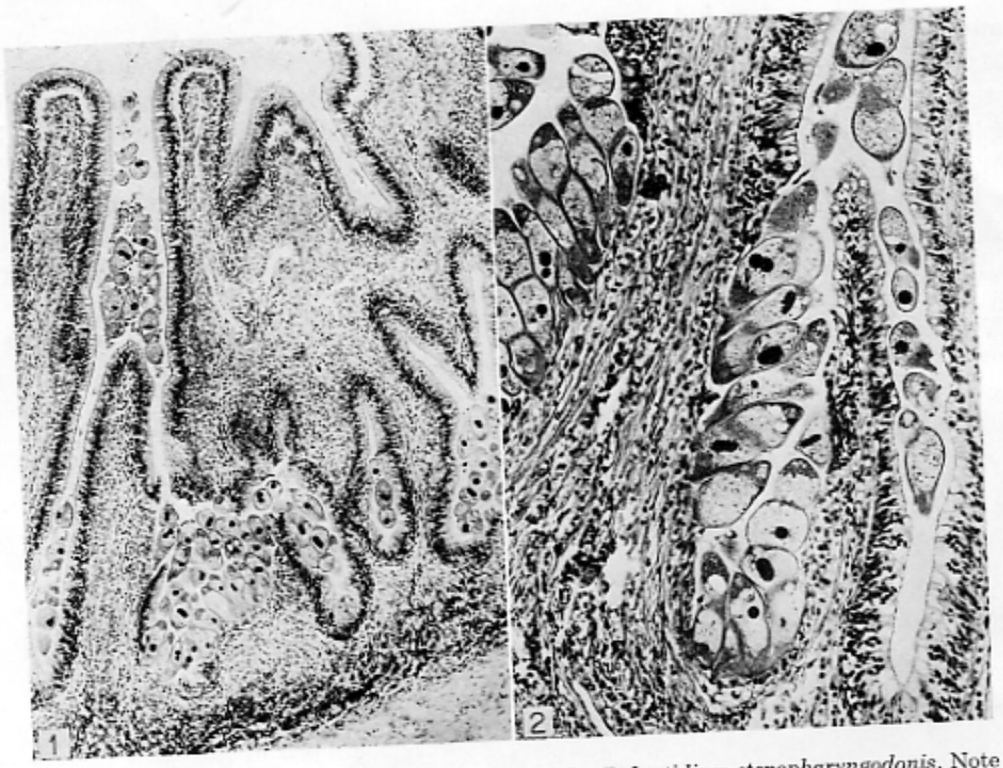

Figure 1. Hindgut of a grasscarp massively infected by *Balantidium ctenopharyngodonis*. Note masses of balantidia in the spaces between the mucosal folds (H & E, ×100).

Figure 2. Balantidia localized in the grooves between mucosal folds. The superficial epithelium is more or less intact at right and at top, but in the other areas the parasites are attached to the propria (H & E, ×200).

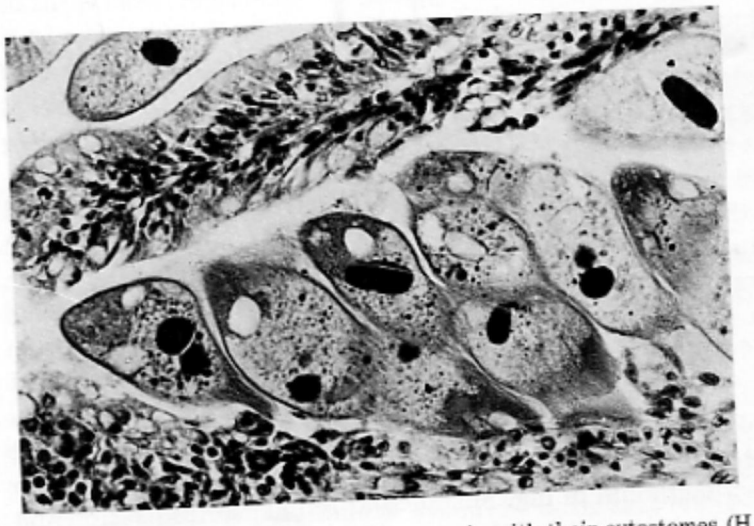

Figure 3. Balantidia adhering to the propria with their cytostomes (H & E, ×500).

of mildly infected and even of non-infected fishes, but there was no epithelial damage. Mention should also be made of the occurrence of light infections of *Spironucleus* in several grasscarp lightly infected or uninfected by balantidia. Spironucleosis is a common flagellate parasitosis of one- and two-summer grasscarps, and may occasionally be lethal (Molnár 1974).

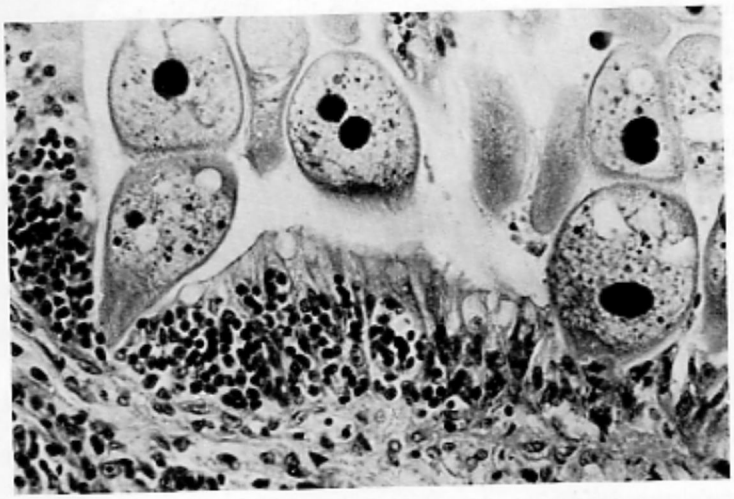

Figure 5. Balantidia in the process of penetrating the intestinal epithelium (H & E, ×500).

Discussion

The present findings indicate that *Balantidium ctenopharyngodonis* generally infects two-summer and, particularly, older grasscarp. The route of infection by balantidia is probably connected with the phytophagous habit of the host and, in accordance with Musselius' (1969) theory, the intake of large amounts of plant food seems to promote the multiplication of the parasite. Usually balantidia can be regarded as innocuous lumen dwelling organisms, the numbers of which become considerably reduced after the conclusion of active feeding. Less often, however, the intensity of infection remains unchanged after the cessation of feeding, as shown in the present studies, and the pathogenic effect of the parasite becomes evident under the influence of certain as yet unidentified predisposing factors.

According to Levine (1961), the pig parasite *Balantidium coli* is a commensal organism, which localizes in the intestinal lumen and derives nourishment from digested intestinal contents and intestinal bacteria, because it is not normally capable of penetrating the intact intestinal mucosa. However, factors depressing the resistance of the host enable the parasite to invade the mucosa and cause ulceration. Enteritis and intestinal ulceration due to balantidiosis was reported by Dickmans (1948) in the dog and by Bogdanovich (1955) in the rat.

In our experience, balantidiosis is also manifested by enteritis and ulceration in

the grasscarp. The ulcers found between the mucosal folds at the sites of parasite invasion had clearly been caused by balantidia. Excessive multiplication of the balantidia could also account for the appearance of the catarrhal exudate which filled the lumen of the posterior part of the gut, while injected vessels in the propria and infiltration of the submucosa by round cells were probably unrelated to balantidiosis. Since the inflammatory changes were also present in those gut portions which had not been invaded by balantidia, there is reason to postulate that the hyperaemic change was due to some primary causal factor, which was also responsible for the excess multiplication of the parasites. In this respect changes in the intestinal bacterial flora may be responsible. It may be significant that *Aeromonas punctata*, a highly pathogenic bacterium was isolated from diseased fish.

We believe that by analogy with mammalian balantidiosis, grasscarp balantidiosis is also a multifactorial disease manifested by catarrhal enteritis and ulceration.

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