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Myxobolus nodulointestinalis Intestinal Parasite of *Barbus* Fishes in Khozestan, Iran

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Abstract: A myxosporean, *Myxobolus nodulointestinalis* has been found in the intestinalis walls of *Barbus* fishes (*Barbus grypus*, *B. sharpeyi*, *B. esocinus*, *B. barbulus* and *B. pectorials*), from Karun and Karkhe Rivers (Khozestan, Iran). Large myxobolus cysts containing mature spores were located in the smooth muscular layer of intestinal wall. The spores had an elongated oval or trapezoid shape. The cysts were branched and separated by septa. They bulged deep into the lumen of the gut and the abdominal cavity. Plasmodia showed an affinity to smooth muscle cells and were covered by a degenerated layer of muscular elements. Because of commercially importance of *Barbus* fishes for pond culture in Iran, this parasite can be acquiring economic importance in aquaculture.

Key words: Myxobolus, *Barbus*, histopathology, parasite, Iran

INTRODUCTION

Of the 500 Myxobolus species know at present (Molnar, 2002), the majority of which were reported from Eurasia and North America (Masoumian *et al*., 1996b). The Khozestan, southwestern region of Iran, is populated mostly by endemic fishes including a wide variety of *Barbus* species. Barboid fishes, among them *Barbus grypus*, *B. sharpeyi*, *B. esocinus*, *B. barbulus* and *B. pectorials* are economically important species in that region. The first report about the myxosporean fauna of these fishes came from Herzog (1969) who described the occurrence of *Myxobolus muelleri* and *M. oviformis*. Subsequently, Al-Salim (1986) and Rashid *et al*. (1989) reported the occurrence of *Myxobolus pfeiffen* in different *Barbus* species. Ebrahimzadeh and Kaylani (1976) and Moghainemi and Abasi (1992) recorded some *Myxobolus* spp. from the internal organs of fishes in the Karun River, Masoumian *et al*. (1994, 1996 a, b) detailed description of *M. karuni*, *M. persicus* and *M. nodulointestinalis* from the gill of *B. grypus* and intestine of *B. sharpeyi*. This study reports the occurrence of *Myxobolus nodulointestinalis* in the gut wall in *Barbus* fushes from Khozestan along with histological evidence on the location of the parasite and the pathologic effects caused by it.

MATERIALS AND METHODS

The studied were conducted in Khozestan province and sample originated from four different sampling site of Karun and Karkhe Rivers (Fig. 1) then the live fish were

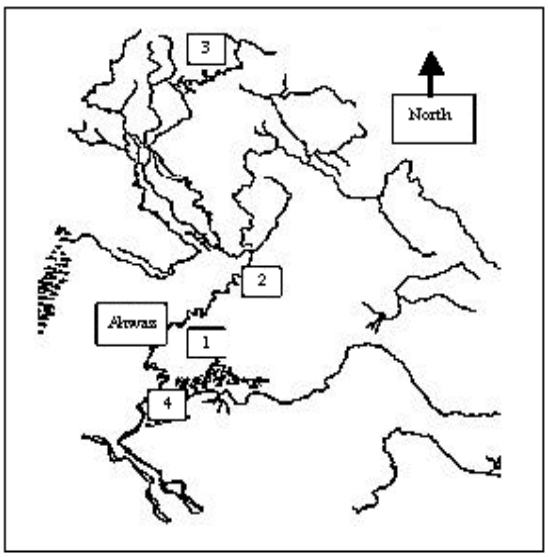

Fig. 1: Sampling site of *Barbus* fishes in Khozestan: 1) Ahwaz-Golestan 2) Karun-Mollasani 3) Karkheh-Hamidiyeh 4) Shadegan marsh

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transported to the laboratory. A total of 296 specimens of 5 species (*Barbus grypus*, *B. sharpeyi*, *B. esocinus*, *B. barbulus* and *B. pectorials*) were examined. The *Barbus* were subjected to complete parasitological examination that included studying Myxobolus infection of the gut. Fishes being killed by transection of the spinal cord and were examined for myxosporean parasites macroscopically and under stereo and light microscope. To avoid shining 0.65% saline solution was sprayed on to the intestinal mucosa in a thin layer and top lighting was applied to search for nodules. From the plasmodia located within the nodules the spores were sucked out with a pipette. On the average 30 spores were measured using the dimensions recommended by Lom and Arthur (1989). Permanent preparations were made by placing a portion of the spores in glycerol-gelatin and mounting them under coverslip. The structure of the polar capsules and the iodophilous vacuole were studied by Nomarski interference microscopy. For histological examinations, small samples were excised from the infected intestinalis segments and fixed in 10% buffered formalin. From the paraffin embedded blocks 5 μm thick sections were made by cutting through the intestine and the sections were stained with Haematoxylin and Eosin.

RESULTS

One hundred and thirteen specimens (38%) of sampling fishes were infected by large white-yellowish Myxobolus cysts in the intestinal wall (infection were seen in member of all 5 species), these cysts showed a branch like structure (Fig. 2). The shape of the spore is relatively constant. It is a comparatively large, elongated ovoid, trapezoid in character, narrower at the posterior end in frontal view, lemon shaped in lateral view, with a protruding structural edge, a distinct sutural line and small indistinct inter capsular appendix. Spore valves are symmetrical and smooth, wall of the spore seems to be very thick but this thickness comes from the emerging sutural edge. Spores 12.6 (11-13) μm long, 8.1 (7.8-9.1) μm wide and 6.3 (5.2-7.2) μm thick. Two polar capsules, pyriform in shape, equal in size, 6.8 (5.2-7.2) μm long, 2.4 (2.2-2.6) μm wide. Polar capsules smaller than the half length of the spores. Anterior ends of the polar capsules set apart from each other. Polar filaments closely coiled with 4-5 turns, situated perpendicular to the longitudinal axis of the capsule. There is a large distinct iodophilous vacuole in each sporoplasm (Fig. 3).

In a cross section of the infected gut segments (Fig. 4) it was clearly visible that the cyst formed in the gut wall bulged deep in to both the intestinal lumen and the abdominal cavity on account of its size. The cyst portion protruding in to the gut lumen pushed the mucosa towards the lumen, the mucosa had markedly narrowed, as had the underlying propria and muscular layer covering the cyst. The other parts of the cyst, covered by the tela

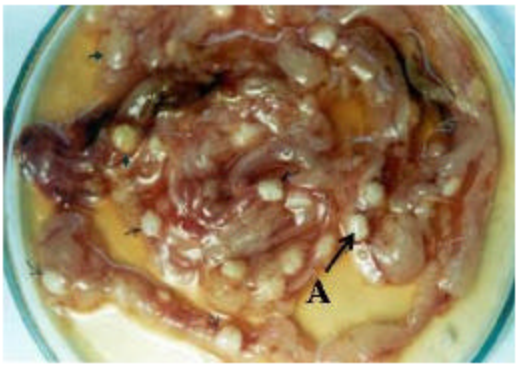

Fig. 2: Large, branched cysts (A) in the intestinal wall of *B. sharpeyi*×1.5

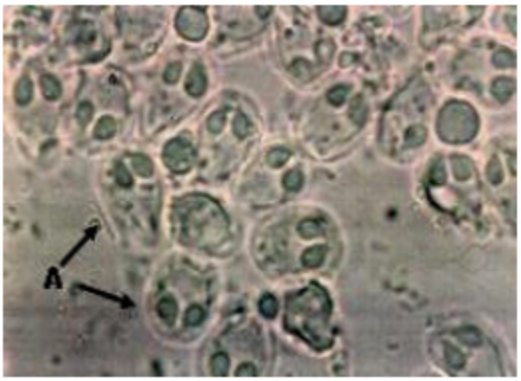

Fig. 3: Unfixed spores (A) of *M. nodolointestinalis* ×1500

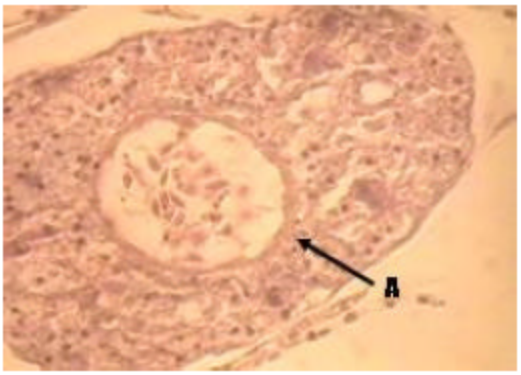

Fig. 4: Cyst (A) in the smooth muscle layer of the intestinal wall of *B. sharpeyi*×1500

subserosa and the serous membrane, bulged in to the abdominal cavity. The cysts were located in the smooth muscle layer of intestinal wall and in some segments of the intestine, branched cysts closely associated with each other and separated by a thin cyst wall were found. The plasmodium was covered by a degenerated layer of smooth muscle. The ectoplasm of the plasmodium consisted of a layer with dark-staining nuclei of generative cells, while the centre of the plasmodium was filled with spores.

DISCUSSION

Barboid fishes are common inhabitants of the rivers of southwest Iran. In identification of the *myxobolus* sp. we found, we accepted Molnar's (1994) theory that the majority of *Myxobolus* species have a relatively strict host specificity which is restricted to closely related fishes. Therefore, during our studies, the *Myxobolus* sp. found in Barboid fishes of Khozestan province was compared with known *Myxobolus* spp. which has been described from the genus *Barbus* or from related genera. In the present case, the reason clearly show that *M. nodulointestinalis* is typical parasite of the intestine and starts its development in the smooth muscle layer, where it forms large cysts. The gut relatively rarely serves as a typical location for the different *Myxobolus* species. It is likely that technical books (Shulman, 1966; Chen and Ma, 1998) erroneously indicate the intestine as a location of infection for several species and has only been recorded as such due to the fact that spores are excreted via the gut. Despite the above probability there are several examples of the infection of *Myxobolus*-plasmodia in the intestinal wall. Some of the cysts showing a branch-like character were possibly formed by the fusion of several neighbouring cysts. By the shape and size of the spores, *Myxobolus nodulointestinalis* distinctly differs from other Myxobolus species known from the intestine of barboid fishes (*M. ellipsoides*, *M. impressus* and *M. obpyriformis*). Major changes in the identification of *Myxosporea* species can be anticipated in the near future. Species descriptions based merely on the morphological characteristics of spores are inadequate and besides the determination of the typical host, host groups and intrapiscine locations, molecular biological identification will also become indispensable. It seems likely that in the future, following accurate re description of the different *Myxobolus* species, determination of their organ and tissue specificity and DNA analysis, many parasites currently regarded as valid species will prove to be synonyms. At the same time, it is also probable that species erroneously identified as synonyms from taxonomically distant hosts based upon the morphological similarity of the spores will prove to represent separate species and add to the number of existing Myxobolus species. *Myxobolus nodulointestinalis* appears to be a pathogenic species. The morphological changes produced in the intestinal wall are accompanied by functional disorders. In the infected portions of the intestine, the gut lumen had become constricted and the wall thickened. From these symptoms it could be determined that the spores released from the matured cysts broke either into the gut or into the abdominal cavity. Both paths of cyst eruption suggest a fatal prognosis. A similar symptom was reported by Egusa and Nakajima (1981), who described intestinal giant cystic disease of the common carp caused by *Thelohanellus kitauei* however, according to Rhee *et al.* (1990) this parasite forms cysts in the intestinal mucosa. While *T. kitauei* is a disease-causing agent characterized by high pathogenicity, the economic importance of *M. nodulointestinalis* is only supposed. *Barbus* fishes are one of the species selected for pond culture in Iran. On that basis, it cannot be excluded that the barboid fishes will acquire economic importance in the near future.

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