Short Paper

Microsporidian infection in lizardfish, *Saurida undosquamis* of Persian Gulf

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Summary

Lizardfish is one of the economically important fishes of Persian Gulf. In recent years, white, ellipsoid, round or elongated nodules were found in body cavity of this fish species which in preliminary microscopic examination were recognized as microsporidia. To determine the approximate prevalence rate of microsporidian infection and to establish its taxonomic position, 50 lizardfish were bought from the local markets of Ahvaz city (the center of Khozestan province – Iran) and transferred to the laboratory for parasitological examination. In the laboratory, internal organs including liver, kidneys, spleen, intestines, gonads and muscles were examined grossly and microscopically for the microsporidian infection using wet and dry smear (stained with Giemsa). Histopathological sections were prepared from the cysts of infected fishes and stained with haematoxylin and eosin to see the arrangement of the spores within the cysts. Some of these small cysts were sampled and fixed in 3% glutaraldehyde for electron microscopic study. According to the results, the total infection rate was 44%. The infection rate in the peritoneum, stomach, gonads, intestine, spleen, muscles and liver were 16, 2, 4, 8, 2, 10 and 2%, respectively. The cysts were mostly ovoid in shape with mean size of 4.3 ± 1.8 mm (0.8 to 10 mm). The spores were ovoid and uninucleate with mean diameter of 2.4×1.3 µm. Polar tube coiled between six and eight time, in one row. According to the histopathology and light and electron microscopic studies, the parasite was recognized as *Glugea sp*.

Key words: *Glugea* sp., Electron microscope, Lizardfish, Persian Gulf, Microsporidia

Introduction

Microsporidia are minute, unicellular organisms living as obligate intracellular parasites in a variety of animal hosts (Didier *et al*., 2004). To date, approximately 1200 species of Microsporidia have been identified and most of these organisms infect invertebrates and fish (Lom *et al*., 1995; Leiro *et al*., 1999; Lee *et al*., 2002). Fourteen species of Microsporidia are known to infect humans. The taxonomy of these organisms has been based primarily on life cycle and ultra-structural characteristics including the size of developing and mature organisms, nuclear arrangement, number and alignment of polar filament coils, intracellular location, and modes of nuclear and cellular division (Wittner and Weiss, 1999).

Fish Microsporidia are embedded directly in the cytoplasm of the host cell which they actually destroy or they induce enormous hypertrophy of the cell (Lom and Nilsen, 2003). The genus *Glugea* (Glugeidae Thelohan, 1892) contains many closely related microsporidian of fish and was placed together with *Loma* in the family Glugeidae by Sprague *et al*. (1992).

Definitive diagnosis of microsporidiosis

still relies upon the detection of organisms. Transmission electron microscopy (TEM) has been the standard method for identifying Microsporidia based on observing the polar filament in the organisms (Terry *et al.*, 1999; Weber *et al.*, 2000). TEM is still important for observing and describing the ultra-structural features of developing and mature organisms that are important in diagnosis (Weber *et al.*, 1999, 2000). However, recently PCR-based methods are also being developed and increasingly applied to improve the sensitivity and species-specificity for diagnosing microsporidiosis (Garcia, 2002).

The most common hosts of Microsporidia are arthropods and fish. In the present study, an unidentified microsporidian infection was found in the viscera and musculature of lizardfish. Lizardfish is one of Synodontidae member that have cylindrical body shape and inhabit benthic biotopes (Golani, 1993). There is no report about Microsporidia infection in this fish species and other fishes of Iran and the Persian Gulf region. The microsporidian *Pleistophora oolyticus* (Negm-Eldin, 1992) have been reported in lizardfish of Egyptian coast of the Red Sea (*Saurida tumbil*). Light and electron microscopy showed that the spores of microsporidian recognized in this study were similar to the genus *Glugea*. However, the size range and the number of polar tube coils in lizardfish microsporidian are different from previously reported species. Microsporidian infections in lizardfish have not been reported previously in Persian Gulf. Since most of fish microsporea are very host specific, the present microsporidian is thought to represent a new species. In our study, morphological and electron microscopic analyses were conducted on this parasite in an attempt to establish its taxonomic position and to determine its prevalence in this fish species in the region.

Materials and Methods

Fifty lizardfish *Saurida undosquamis* (500-1400 g) were bought from the local markets of Ahvaz, Iran, and transferred to the Fish Health Laboratory of Veterinary Faculty. In the laboratory, internal organs including liver, kidneys, spleen, intestines, gonads and muscles were examined for the microsporidian infection. The cysts were grossly observable and easily collected from the infected fishes.

Light microscopic study

Wet squash smear was prepared from each organ and carefully observed under the microscope. The cysts from the infected organs were dissected and opened in normal saline and thin smears were prepared from the cyst fluid. The smears were let to dry at room temperature and fixed in methanol. After drying, the smears were stained with Giemsa, (1:10 dilution for 20 min).

Histopathologic sections were also prepared from the cysts. For this purpose some of cysts were fixed directly in 10% buffered formalin. Histopathologic sections were prepared from the cysts by routine methods to see the arrangement of the spores within the cysts.

Electron microscopic study

Some of the smallest target cysts were separated from the tissues and primarily fixed with 3% glutaraldehyde in phosphate buffer. The cysts were used in blocks for transmission electron microscopy (TEM) processing. The blocks were post fixed in 1% osmium tetroxide for two h and washed with phosphate buffer. The blocks were dehydrated in the graded series of acetone. The selected specimens for TEM investigation were infiltrated in different concentrations of resin in acetone and embedded in beam capsules filled with 100% resin. Ultrathin sections (60-80 nm) were prepared on the ultramicrotome and placed on copper grids which were subsequently stained with uranyl acetate and lead citrate and examined under the TEM (Leo model 909). Cysts were also prepared for scanning electron microscopic study (SEM) (SC7620 sputter coater – Leo 1455 VP SEM, Germany) by routine methods.

Results

The infected lizardfish were apparently normal, without any external symptom of

disease. The microsporidia had affected 22 individuals of 50 lizardfish, resulting in 44% infection rate. Grossly, groups of xenomas often formed white masses approximately 5 mm in diameter in various visceral organs (Fig. 1). Individual xenomas diameter ranged from about 0.8 to 10 mm. In stained dry smears, the diameters of mature spores were estimated as 1.3×2.4 μm (Figs. 2 and 3). The xenomas mostly affected the peritoneal cavity, but also occurred in spleen, liver, gonads, kidney, intestine and in the skeletal muscles. According to the results, the total infection rate was 44% and the infection rate in the peritoneum or body cavity, stomach, gonads, intestine, spleen, muscles and liver were 16, 2, 4, 8, 2, 10 and 2%, respectively. The cysts were mostly ovoid with mean size of 4.3 ± 1.8 mm.

In histological sections, large numbers of spores were seen within the cysts which were surrounded by a dense connective tissue. The cyst seems to be the overgrowth of the infected cells which induced by microsporidia multiplication. These large xenomas had the walls consisted of thick connective tissue (Fig. 4).

In ultra-microscopic study, large numbers of spores were observed within the cysts. The spores were ovoid and uninucleate with mean size of 2.4×1.3 μm. Polar tube coiled between six and eight time, in one row (Figs. 5, 6 and 7).

According to the morphology of parasite in histopathological sections, light and electron microscopic studies of spores, and also according to identification keys (Lom, 2002), the parasite was identified as *Glugea sp*. The spores of all cysts were similar; therefore, there is no doubt that in all cases of this study, the cysts were identical and caused by one species (*Glugea sp.*).

Discussion

Due to species diversity, classification and determination of fish microsporidian species is very difficult. Most of reports on these parasites in the world have been done at the genus level and there are a lot of controversies about the reported species (Lom and Nilsen, 2003). Because of many problems in diagnosis of species in this point

we prefer to report this parasite at the genus level. This is the first report of microsporidian parasite in Iran. However, for definite diagnosis of this parasite at the species level, very complicated steps should be done. This microsporidian species seems

Fig. 1: One of the heavily infected lizardfish seen in our study. Body cavity of fish as well as the viscera is covered by the microsporidian cysts
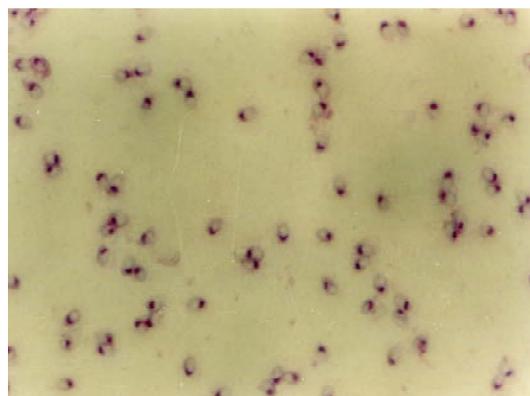

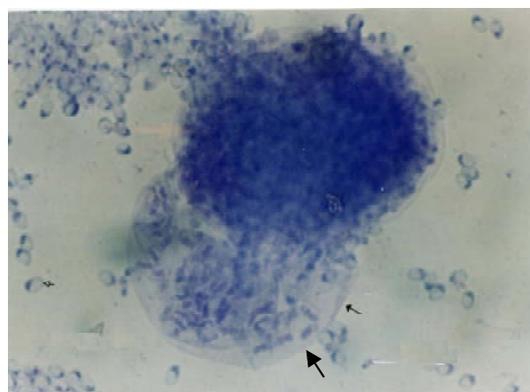**Fig. 2: A large number of spores in thin smear (Giemsa staining, ×1000)**

Fig. 3: Some of the spores have been encapsulated in a thin layer (arrows), (Giemsa staining, ×1000)
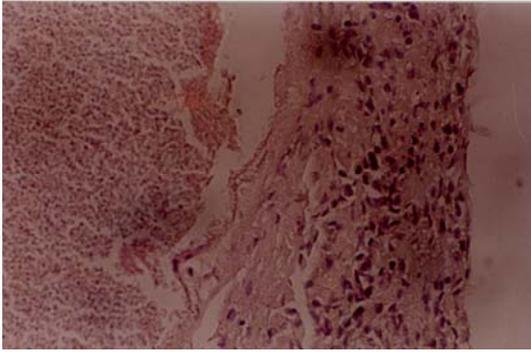
Fig. 4: A histological section of the cysts. The thick layer of connective tissue (right side of picture) can be seen around the spores (left side of picture) (H&E, ×400)
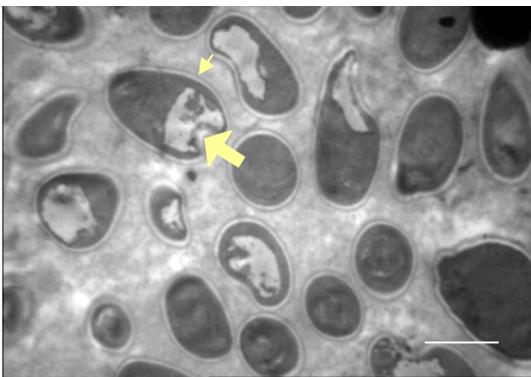

Fig. 5: Mature spores in TEM section. Posterior vacuole (large arrow) and the spore wall (small arrow) can be seen, (Bar = 1.53 µm)
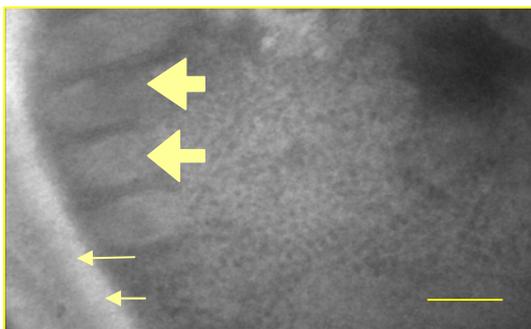

Fig. 6: Mature spores in TEM section. Polar tube (large arrows) and one layer spore wall (small arrow) can be seen, (Bar = 0.11 µm)

to be a new species and its characteristics did not match completely with previously reported species. To introduce it as a new species we need to send it to a reference laboratory. However, the pattern of cyst formation and spore structure are characteristics of the commonly reported *Glugea* group, *G. anomala* (Canning *et al*.,
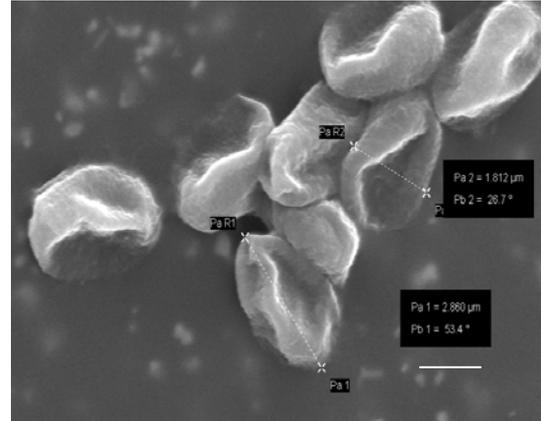

Fig. 7: Mature spore in SEM sample, (Pa1 and Pa2 = Spore diameters, Pb1 and Pb2 = spore angles), (Bar = 1.1 µm)

1982), *G. weissenbergi* (Sprague and Vernick, 1968; Takvorian and Cali, 1983), *G. atherinae* and *G. plecoglossi* (Lom, 2002).

According to the results, the total infection rate was 44% and large numbers of spores were produced within the cysts of different sizes. Such a macrospore infection has not been found in other fish species of Persian Gulf, therefore, this parasite seems to be a host-specific parasite, but further researches are needed to confirm this result. Many microsporidian species infect only one specific host, while others may readily infect a variety of hosts (Moura *et al*., 1999; Trammer *et al*., 1999; Lowman *et al*., 2000). Recently, the low host specificity of some microsporidians has been demonstrated and it has been indicated that many of these micro-organisms could be transmitted even from invertebrates to mammals and are able to adapt to changes in temperature (Lores *et al*., 2003). No microsporidian infections in lizardfish have been reported previously in Persian Gulf. Consequently, the present microsporidium is thought to represent a new species.

In microscopic study of the current investigation, large numbers of spores were observed within the cysts of different sizes. The cysts walls consisted of thick connective tissue. Hypertrophic growth of the infected cells was induced by microsporidia, which results in formation of large xenomas. Early thin-walled xenomas did not elicit any reaction of the surrounding tissue. Later, however, coinciding with the

mature xenoma being filled up with a growing mass of spores, proliferative inflammatory reaction occurred and xenoma gradually changed into a granuloma. Essentially, the tissue reaction was found to follow the pattern known in *G. anomala* infections in sticklebacks and in some other *Glugea* species initiating the formation of large xenomas (Dyková *et al*., 1980).

The spores were ovoid and uninucleate with mean size of 2.4 × 1.3 µm and with one layer wall that in some cases was shrunken. Polar tube coiled between six and eight time, in one row. The *Glugea*-type is represented by thin walls, at first appeared as unit membrane-like structures and later modified and thickened slightly. The spore wall usually has a smooth surface, but may shrink under the effect of fixatives into low irregular wrinkled wall and creases especially when viewed under the scanning electron microscope (e.g. *Glugea hertwigi*, *G. weissenbergi*). According to light microscopy, *Glugea* recognized in this study has spores similar to *G. anomala*. However, the size of *G. anomala* spores as given by Canning *et al*. (1982) is 1.9-2.7 × 3-5.6 µm and the number of polar tube coils in *G. anomala* is about 12 to 14.

In conclusion, lizardfish of the species *Saurida undosquamis* was infected by a microsporidian of the genus *Glugea*. In various body organs, primarily in the peritoneal wall, it induced the formation of xenomas with a typical structure of *Glugea* spp. The xenoma structure and spore morphology assigns them to the genus *Glugea*. Several other authors (e.g. Canning *et al*., 1982) have reported similar parasites, but unfortunately their descriptions were inadequate for identifying the parasite at the species level. However, because the host is a different species from the previously reported fish species, its microsporidian may be a new species. Therefore, for introducing the present parasite as a new species, further studies are needed.

Acknowledgement

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