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BIOLOGY AND HISTOPATHOLOGY OF *THELOHANELLUS HOVORKAI* ACHMEROV, 1960 (MYXOSPOREA, MYXOZOA), A PROTOZOAN PARASITE OF THE COMMON CARP (*CYPRINUS CARPIO*)

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Thelohanellus hovorkai*, a protozoan introduced in Hungary from the Far East, is a frequent parasite of one- and two-summer common carp reared in fish ponds. The plasmodia are demonstrable most frequently in the summer months, in the buccal tissues of the head of fry. In one-summer common carp, plasmodia occur more frequently on the swimbladder serosa. A certain seasonality can be observed in the occurrence of *T. hovorkai;* namely, infection appears in early summer and disappears by the end of summer. The first developmental stages were demonstrable in 4 weeks old fry; in 6 to 8 weeks old fish mostly cysts containing only spores occurred. *T. hovorkai* is a typical connective-tissue parasite; its development is always associated with the dense connective tissue. The cysts of *T. hovorkai* are 0.3 to 0.5 mm in diameter, are surrounded by one or two rows of connective-tissue cells, and most frequently sit on ligaments, fasciae or on the adventitia constituting the walls of blood vessels.

Keywords. *Thelohanellus hovorkai*, Protozoa, parasite, biology, histopathology, common carp (*Cyprinus carpio*).

In an earlier paper (Molnár and Kovács-Gayer, 1981–82) we reported the occurrence in Hungary of two common carp parasite *Thelohanellus* species introduced from the Far East, *Thelohanellus nikolskii* Achmerov, 1955 and *T. hovorkai* Achmerov, 1960. Of these two species, the biology and histopathology of *T. nikolskii* were described in detail by Molnár (1982), and its ultrastructure by Desser et al. (1983).

In the present paper our observations made on the biology, pathomechanism and tissue specificity of *T. hovorkai* during the dissection and histological examination of common carp reared in pond farms are reported.

Materials and methods

Common carp from the fry-rearing and rearing ponds of different Hungarian fish farms were used. The investigations were started in 1980, when the parasite was first demonstrated histologically in common carp submitted for routine laboratory examination. In 1981 and 1982 mainly two-summer fish were examined, but in 1982 to 1984 the investigations were extended to the fry as well. At two-week intervals samples were taken from the fish ponds that had been found infected in earlier years, and the sampled fry were examined by dissection continuously. Results on two-summer fish came from the processing of fish sent for routine laboratory examination.

Infection was monitored by light-microscopic examination of squash preparations made from the organs and smaller parts of the fish (the organs and body parts were squashed between glass-plates). When spores or cysts were present, the infected organs were fixed for histological examination. In 1983 and 1984, 2- to 3-cm-long fry from consistently infected fish ponds were fixed in their entirety, without previous examination.

For histological fixation, 10% neutral formalin or Bouin's solution was used. After embedding in paraffin, 4 μm thick sections were made from the organs, and from fry cleaved in the longitudinal plane of the body. The sections were stained with haematoxylin and eosin, and according to Farkas–Mallory's and van Gieson's technique.

Results

Observations

In a histological section, a *Thelohanellus hovorkai* cyst was first observed in 1980, on the swimbladder serosa of a two-summer common carp. In 1981 further cysts were found in two-summer fish in the subserosal connective tissue of the swimbladder, and such cysts occurred also in the connective tissue of the gill arch. In that year cysts were found on the swimbladder in native state, and the examination of spores obtained from the cyst rendered possible a precise definition of the parasite species. In 1982 it became clear that *T. hovorkai* was a frequent parasite of certain fish farms; intensive infection occurred mainly in fry. Investigations conducted between 1982 and 1984 revealed that the first cysts occurred already in one-month-old, 2 to 2.5 cm long fry, and in 6 to 8 weeks old fish even spore-containing cysts were demonstrable. In some stocks the infection rate reached 80 to 90%. In the majority of cases, *T. hovorkai* infection occurred jointly with *T. nikolskii* infection. The clinically apparent *T. nikolskii* infection was pathognomic since it occurred synchronously with *T. hovorkai* infection, which could not be diagnosed solely by exterior examination. By native examination *T. hovorkai* infection was easiest to demonstrate from the head of the fish. Under a stereomicroscope the small, pinhead-sized cysts 0.3 to 0.5 mm in diameter could be released from the buccal tissues with a dissecting needle. The cysts contained relatively few, most frequently 200 to 400, spores. As a rule, infection ceased to exist by the end of August. In September only a few scattered spores were found in the melanomacrophage centres of the kidneys and spleen. No clinically apparent disease or deaths were observed in intensively infected stocks either.

T. hovorkai infection did not occur in grasscarp, silver carp and bighead stocks kept in the infected fish ponds.

Histological studies

In fry, *T. hovorkai* cysts occurred most frequently in the periorbital loose connective tissue (Fig. 1) and around the nose; however, cysts were of frequent occurrence also in the operculum, among the muscles bordering the gill-opening (Fig. 2) and in the perirenal connective tissue. Less frequently, solitary cysts were demonstrated also in the gill arch, among the tail muscles, and in the connective tissue surrounding the vertebral column. In two-summer fish, cysts were found in three locations: most frequently in the subserosal connective tissue of the swimbladder and in the compact connective tissue covering the gill arch cartilage. However, on one occasion a cyst containing numerous spores was found under the serosa of the extrahepatic pancreas lying on the intestinal wall, embedded in the pancreatic substance.

The plasmodia always started to develop among connective-tissue cells, irrespective of the organ in which they developed. They showed pronounced affinity for the compact connective tissue; therefore, even the cysts situated in the loose connective tissue frequently sat on ligaments, fasciae, or on the adventitia constituting the wall of blood vessels (Figs 1, 4 and 5). Among the muscles the connective tissue separating muscle fibres, whereas in the case of the serous membranes the connective tissue lying beneath the coelothel of the serosa was their site of establishment. In parenchymal organs no plasmodia occurred except those lying beneath the serosa covering these organs; however, after the disruption of cysts that had developed in other parts of the body, one or two spores were frequently demonstrated in the melanomacrophage centres of the above organs. *T. hovorkai* plasmodia were surrounded by a rather thin capsule consisting of one or two rows of connective tissue cells. The youngest plasmodia observed by us were 16 to 20 μm in diameter and approximately round in shape (Fig. 3); within them, a 4-μm-thick ectoplasm and an endoplasm constituted by 12 to 20 cells were distinguishable. The plasmodium was surrounded by connective-tissue cells having relatively large nuclei. The plasmodia showing a more advanced stage of development and containing pansporoblasts and immature spores were also spherical in shape. Within the cyst 80 to 120 μm in diameter, the 8 to 10 μm thick, eosinophilic ectoplasm and the endoplasm were well-distinguishable (Fig. 4). The cysts, attached with one pole to the adventitia of blood vessels or to other compact connective tissues, were separated from the surrounding loose connective tissue by a thin capsule. The mature, spore-containing cysts were mostly oval or ellipsoidal in shape and reached a size of 160–200 $\times$ 70–110 μm in fry (Fig. 5) and even 300 $\times$ 140 μm in two-summer common carp. Their ectoplasm disappeared, the connective-tissue cells covering the cyst became flattened. Beside the loose connective tissue, the cyst wall was frequently contiguous with the coelothel, muscle cells or parenchymal cells; however, the compact connective tissue indicating the origin was present on one of the poles of the cyst also in these cases (Fig. 5).

Discussion

The present studies indicate that *T. hovorkai* is a common carp parasite widespread in Hungary; it is at least as frequent as the fin parasite *T. nikolskii.* Since the plasmodia of *T. hovorkai* are relatively small and develop in the tissues, without microscopic examination it is difficult to diagnose the infection, which frequently remains unnoticed. Obviously, the parasite was introduced into Hungary from the Far East, similarly to *T. nikolskii* (Molnár and Kovács-Gayer, 1981/82) and has become widespread among common carp cultured in pond farms. According to our investigations, the infection is most frequent in the young fry; however, it occurs in two-summer fish as well. *T. hovorkai* is a connective-tissue parasite; thus, it may occur in all body parts which are rich in connective tissue. In fry, the most frequent location of the parasite is the connective tissue separating the muscles of the head and those around the pharynx, while in older fish the parasite was demonstrated in the connective tissue of serous membranes and gill arches most frequently. A more precise localization of the infection in older fish was hampered by the fact that in fry the location was determined by processing and examining all organs, whereas in two-summer fish organs less important from the aspect of routine examinations were not always studied.

Therefore, it cannot be excluded that in older fish infection occurs also in places other than the gills and serous membranes. The development of *T. hovorkai* shows a yearly cycle pattern. Young plasmodia appear in the fish in June or July. Spores are formed within the plasmodia after a developmental period of 4 to 6 weeks, depending on the temperature. In 6 to 8 weeks old fish mainly spore-containing cysts are found. Young cysts are surrounded by connective-tissue cells rich in plasm; later on, parallel to the growth of plasmodia, these cells become flattened and form a thin ring of 1 or 2 cell rows. After the disruption of cysts the 200 to 400 spores get into the neighbouring connective tissue, from where the lymph or blood circulation transports them to various organs. The excretion of spores to the outworld is obviously similar to that described for the muscle parasite *Myxobolus cyprini* by Molnár and Kovács-Gayer (1985); i.e. the spores are expulsed from the capillaries of the gills, skin, renal tubules and intestine to the outworld through local necroses. At the same time, spores stuck and encapsulated in the cyst, or destroyed in the melanomacrophage centres of the spleen and kidneys, were found also in *T. hovorkai* infection.

In the Far East, besides *T. hovorkai* several other *Thelohanellus* spp. parasitize the common carp *(Cyprinus carpio)*. Of these, Achmerov (1960) reported *T. nikolskii* from the fins, *T. dogieli* from the skin, *T. hovorkai* from the peritoneum, *T. acuminatus* from the gill lamellae, and *T. amurensis* from the liver of the common carp. Japanese authors widened the circle of these parasites. Hoshina and Hoshoda (1957) reported the species *T. cyprini* from the fins, while Egusa and Nakajima (1981) *T. kitauei* from the intestinal wall. Although Shulman (1966) considered the majority of the species to be synonymous with *T. dogieli* and *T. furmanni*, based upon spore morphology and the pronounced organo-specificity today it is unquestionable that only *T. cyprini* can be regarded as a synonym of *T. nikolskii*, while the validity of the remaining species seems to be proved. The extraordinary organo-specificity of *Thelohanellus* spp. was first described by Achmerov (1955). Later on, his observation was supported by the results of Molnár (1982) and Molnár and Kovács-Gayer (1981–82) on *T. nikolskii* and *T. hovorkai*. The development of *T. hovorkai* within the fish indicates that in the case of *Thelohanellus* spp. the term "tissue-specificity" should be used instead of organo-specificity.

Although, similarly to Achmerov (1960), we also found *T. hovorkai* first on the serosa, more detailed investigations have revealed that this species occurs in all organs rich in connective tissue. *T. hovorkai* is a connective-tissue parasite, as opposed to the cartilage parasite *T. nikolskii*, whose development is associated with perichondrial cells (Molnár, 1982), or to *T. kitauei* whose typical site of development is the intestinal mucosa. In addition to the tissue-specificity of *Thelohanellus* spp. parasitizing the same host, pronounced anatomical differences existing in spore morphology ensure an accurate species identification. Similarly, the various species are characterized by different host responses. *T. hovorkai* spores, differing from the spores of *T. nikolskii* in their considerably larger polar capsules, develop in relatively small cysts containing significantly fewer spores than those of *T. nikolskii*. In *T. hovorkai* infection, the host response developing around the small cysts and restricted to a connective-tissue capsule consisting of a few cell rows, can be considered negligible as compared to the thick, cartilageous and connective-tissue capsule developing around *T. nikolskii* plasmodia.

Unfortunately, no conclusions on the pathogenicity of *T. hovorkai* can be drawn from the present studies. *T. hovorkai* seems to be a species of low pathogenicity; since it is located in less vital organs, it presumably does not cause deaths among the fish. However, because of its regular and consistent occurrence primarily in fry, its role in decreasing the host's resistance should not be neglected.

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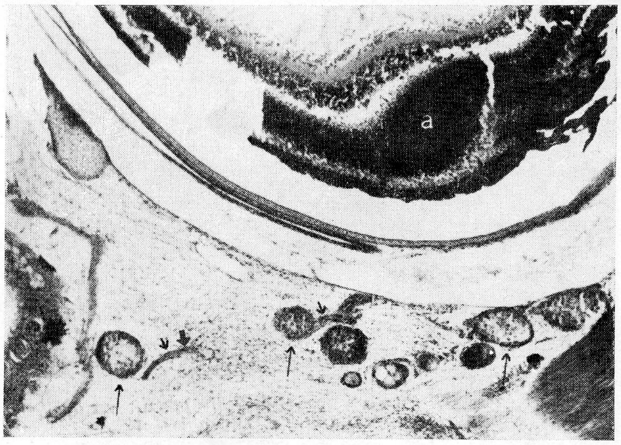

Fig. 1. Thelohanellus hovorkai cysts in the periorbital connective tissue. Orbital cavity (a), cysts (↑), dense connective tissue (↑). Haematoxylin–eosin, ×100

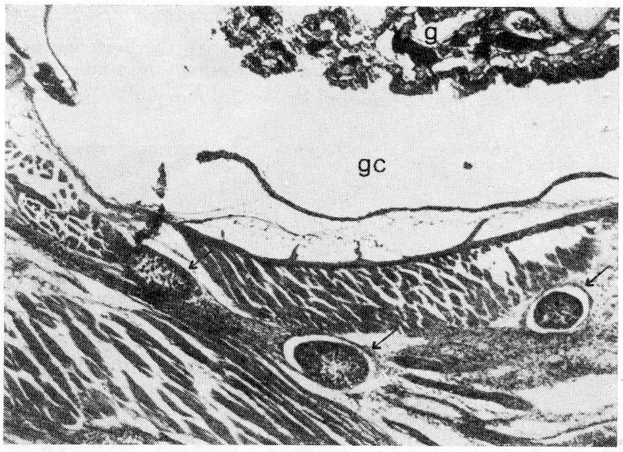

Fig. 2. Thelohanellus hovorkai cysts among the muscle bundles neighbouring the gill cavity. Gill cavity (gc), gill (g), cysts (↑). H. and E., ×100

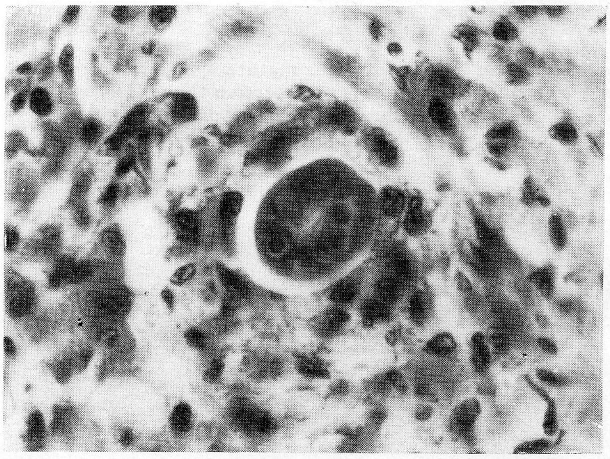

Fig. 3. Young *Thelohanellus hovorkai* plasmodium in the loose connective tissue of the head of a common carp fry. H. and E., ×1200

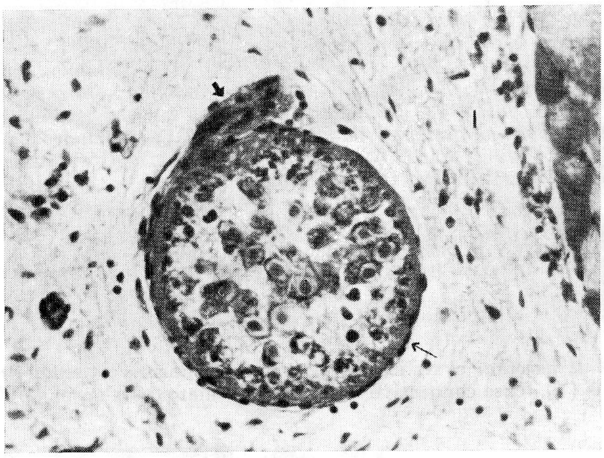

Fig. 4. Developing plasmodium containing young spores in the loose connective tissue. Loose connective tissue (1), dense connective tissue (↑), connective tissue cells constituting the capsule of the cyst (↑). H. and E., ×400

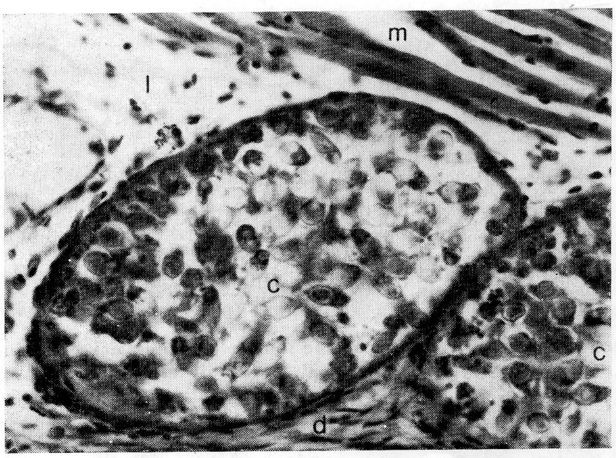

Fig. 5. ***Thelohanellus hovorkai*** cysts containing mature spores in the intermuscular connective tissue of common carp fry. Cysts (c), loose connective tissue (1), dense connective tissue (d), muscle fibres (m). H. and E., ×400