Abstract

The surveys were made in 60 carp farms with special emphasis on Thelohanellus nikolskii detection. The fresh preparations, histological sections and ultra-thin sections were examined. The presence of Thelohanellus nikolskii in carp in Poland has been finally confirmed on the basis of the presence of characteristic cysts and spores on the fins. Our preliminary observation has shown that the parasite does not develop in traditional carp farms but its development is possible in warm water cage farms, where the temperature of the water is by 4 - 6°C higher than that in ponds.

Key words: carp, Thelohanellus nikolskii, epizootiology, pathogenesis.

Results

In 2002 infected with Thelohanellus nikolskii carp yearlings imported from Hungary were put immediately into traditional pond carp and additionally in warm water cage. In 2003 fish in both farms were examined parasitologically twice a year in July and in August.

The cysts which appeared on the fish fins were examined in fresh preparations and histological sections stained with haematoxylin – eosin. The ultra-thin sections of fish fins were prepared also for electron microscopy. The samples were fixed in 2.5% glutaraldehyde buffered with ice-cold 0.1M cacodylate buffer (pH 7.3) for 2-3 h and then washed with the same buffer, containing 3% sucrose, and osmicated with the buffered 1% osmium tetroxide. The tissues were dehydrated by graded series of cold ethanol and propylene oxide, embedded in low viscosity resin (7), cut into ultra-thin sections, doubly treated with uracyl acetate and lead citrate (6) and examined under BS-500 Tessla transmission electron microscope.

Material and Method

In 60 traditional carp farms (earthen ponds) and in 7 cage farms (fed with reused warm water from electricity plant) the surveys were made twice a year with the special emphasis on Thelohanellus nikolskii detection. The monitoring was performed in 2003, 2004 and 2005.

In 2002 infected with Thelohanellus nikolskii carp yearlings imported from Hungary were put immediately into traditional pond carp and additionally in warm water cage. In 2003 fish in both farms were examined parasitologically twice a year in July and in August.
appear on all fins (Figs 1, 2). The extensity of infection in carp yearlings has been up to 80% and intensity up to 65 cysts per fish. The extensity and intensity of infection in two and three year carps were much lower.

In August in some fishes in pond farms the cysts and white round spots in places where previously the cysts had been located were seen. Also sporadic, usually healed, fin lesions could be detected. In the same time in warm water cages no cysts could be seen but in some fishes the broken tail fins were visible (Fig.3), sporadically the tail fins were entirely missing and pathological process proceeded up to fin peduncles (Fig. 4).

In July in traditional earthen ponds where the infected carp yearlings (imported from Hungary) were stocked no parasite cysts were noted in the next year, while in warm water cage farms they appeared again on the fins of two year old carps, however, the intensity and extensity of the infection were very low.

At the beginning of July, inside the cysts proliferating generative cells and vegetative nuclei were present but no spores were seen. As a result of endogenous cell division secondary cells inside primary cells (which is characteristic for Myxosporea development) were produced (Fig. 5).

In the end of July and at the beginning of August the ripe spores appears and they were located in the centre of the cysts (Figs 6, 7). The cyst wall consisted all the normal fish epidermal layers and cortical zone produced by polysporic plasmodium (Fig. 8). Between the epidermal and cortical layer melanophores were present.

The average spores were 15µm long and 11µm wide in the broadest place. Maximal size of the spore was 17×12µm. The majority of spores had one large spherical polar cell 6µm in diameter, sporadically two or three polar cells could be found (Fig. 9). Those atypical spores were much broader than average spore i.e up to 14µm wide. In TEM photos we found that polar filament (inside polar cell) was arranged in a double coil and has 7 turns in the outer and 2 turns in the inner coil (Fig. 10).

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Fig. 1. Cysts of *Thelohanellus nikolskii* on fins of carp yearling.

Fig. 2. Histological section of *Thelohanellus nikolskii* cysts (about 2mm in diameter).

Fig. 3. Broken tail fin caused by *Thelohanellus nikolskii* cysts.

Fig. 4. Secondary bacterial infection of fin peduncle which begins at the site of *Thelohanellus nikolskii* cysts.
Fig. 5. *Thelohanellus nikolskii* developmental stages. Secondary cells inside primary cells. TEM. Bar ___ 1.5 μm.

Fig. 6. *Thelohanellus nikolskii* typical spores: in one end of the spore spherical polar cell with coiled polar filament, in the another sporoplasmic cell.

Fig. 7. *Thelohanellus nikolskii* spore: sporosmic cell inside shell valves. TEM. Bar ___ 1.5 μm.

Fig. 8. *Thelohanellus nikolskii* cyst structure from the outside: fish epidermis, melanophore layer, cortical zone, developmental stages and spores (inside) arrow. TEM. Bar __ 30 μm.

Fig. 9. Atypical *Thelohanellus nikolskii* spore with three polar cells, arrows.

Fig. 10. Polar cell with transverse section of coiled polar filament, arrow. TEM. Bar __ 0.6 μm.
Discussion

The presence of *Thelohanellus nikolskii* in carp in Poland has been finally confirmed. The size and structure of ripe spores and specific cyst location on fish body generally agree with the descriptions presented by Lom and Dykova (4).

The average size of spores determined in our investigations was a little smaller, i.e. 15x11µm, than the size observed by Lom and Dykova (4), i.e. 17x10µm, though maximal size of the spores found in thelohanellosis cases in Poland (17x12µm) was equal to that presented by above mentioned authors. Lom and Dykova (4) observed 8 coils of polar filaments, whereas in our examination 7 coils were found. This difference seems to be not important to exclude the described parasite out of the species *Thelohanellus nikolskii* and could be explained, similarly as occasional appearance of two or three polar cells in spore, as sporadic deviations (mutations).

Thelohanelliosis is a new disease in Polish fish farms and was never diagnosed until 2001 and up to now its locations were restricted to three farms which imported 4 month old carp from Hungary, where the parasitosis is diagnosed each year (5).

The carp yearlings infected with *Thelohanellus nikolskii* in Hungary (cysts in fins) and put to traditional earthen ponds in Poland did not develop thelohanellosis next year testifying that the parasite cycle was interrupted. In the carp from the same group which were placed in warm water cages, the parasite cysts have appeared next year. It was shown, therefore, that the development of *Thelohanellus nikolskii* is possible in this type of the environment also in Poland. The cage carp culture differ from pond carp culture in respect of the intensity of fish production and temperature of water in cage farm which is fed with reused water from electricity plant. The temperature of water during the year is by 4-6ºC higher than that in pond farms fed with river water. The density of fishes is much greater in cages than in ponds. These factors probably facilitate the development and spread of *Thelohanellus nikolskii* in cage facilities.

According to Anderson et al. (1) myxosporean and actinosporean stages of *Thelohanellus hovorkai* develop in common carp and water oligochaete, respectively. It is quite sure that analogous development is taking place in the case of *Thelohanellus nikolskii*. The intermediate host of this parasite was not identified yet but it should be present in the environment of cage culture in Poland where the whole developmental cycle of the parasite could take place.

*Thelohanellus nikolskii* so far induces rather little economic losses and it concerns mainly carp yearlings kept in warm water cages. In this particular environment tail fin lesions do not heal easily because of secondary infection with opportunistic bacteria which abound in water and sediment of warm water reservoirs and therefore in some cases outcome of the disease could be fatal for fish.

References