

DIFFERENT MANIFESTATIONS OF *THELOHANELLUS NIKOLSKII* INFECTION IN CARP (*CYPRINUS CARPIO*)

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Thelohanellosis is a disease that occurs in all carp categories. The cause is protozoa Thelohanellus nikolskii Achmerov, 1955. It manifests itself in two forms. The first form occurs on the fins in one-year-old carp fry and the second one occurs on scales in two-year-old, three-year-old and older categories of common carp (Cyprinus carpio). The researches conducted between 2008 and 2012 covered 22 fish ponds, 18 of which are located in Serbia, and 4 in Bosnia and Herzegovina, Republic of Srpska. It has been concluded that both forms of the disease were present in all observed fish ponds. Prevalence of telohanellosis located on the fins ranged between 3% and 30%, and the infection intensity was between 2 and 86 cysts per individual, while in the case of thelohanellosis on scales the values were higher. Changes on the scales were present during April and May, and on the fins during July and August. After determination of morphological characteristics of spores and histopathological characteristics of plasmodia located on the fins and scales, no significant differences were noticed between them. Also, artificial infestation of carp fingerlings for species differentiation was done.

Since there are no adequate therapeutic measures, control of thelohanellosis is still based on compliance with basic sanitary-prophylactic measures such as drying of equipment, freezing, mechanical cleaning and disinfection with lime.

Key words: carp, fins, plasmodia, scales, Thelohanellus nikolskii

INTRODUCTION

Achmerov (1955) described several species of myxosporean parasites including *Thelohanellus nikolskii* isolated from cysts presented on the fins. The same parasite was described by Hoshina and Hosoda (1957) in carp fingerlings in fish ponds in Japan and named it *Thelohanellus cyprini*. Ivasik and Karpenko (1967) reported that *Thelohanellus nikolskii* entered from the western parts of Ukraine to Belarus by means of wild carp. The same authors reported that wild carp and their hybrids transmitted the parasite via the river Amur to the Trans Siberian area (in 1963). *Thelohanellus nikolskii* infection in Europe was first reported in Hungary by Janey (1979) as cysts found on the fins of carp fingerlings. The parasite was introduced from the far East and causes each year an intensive infection of carp fry in Hungary (Molnar and Kovacs-Gayer, 1981-1982; Molnar, 2002). In Serbia *Thelohanellus nikolskii* was reported in the beginning of the eighties (Ćirković *et al.*, 1983) and is still present. In Moldova, *T. nikolskii* infection was first recorded by Trombitsky *et al.* (1983, 1990). The first cases of thelohanellosis in Poland was described by Antychowicz (2003) and Kramer and Przybysz (2004). In Czech Republic, Novotny and Smolova (2006) was first who reported *Thelohanellus nikolskii* infection of carp fingerlings which were imported in this country.

Wolf and Markiw (1984) were the first who described the life cycle of *Myxobolus cerebralis*, while Yokoyama (1997) and Székely *et al.* (1998) finally described the life cycle of *Thelohanellus nikolskii* and *Thelohanellus hovorkai*. Intermediate hosts are oligochaetes, usually *Tubifex tubifex* in which actinosporean stages transform into the aurantiactinomyxon spores which are infective to fish. Pathological changes caused by *T. nikolskii* infection on the fins were studied by Molnar (1982), Ćirković (1986) and Ćirković *et al.* (1997). Changes on the fins are characterized by the appearance of plasmodia 1-2 mm size (Molnar, 1982; Ćirković, 1986) in one-year-old carp. Ćirković *et al.* (1997) presented the six thelohanellosis phases that occur in carp fingerlings aged 21 to 70 days.

Desser *et al.* (1983) described the ultrastructural characteristics of sporogenesis of *T. nikolskii* from the fins.

The host immune response to the presence of these parasites occurs through cellular immunity, and tissue reaction. Studies showed that the humoral response to myxosporeans is quite low (Griffin and Davis, 1979). Myxosporeans probably avoid the creation of antibodies by the host hiding antigen (Pauley 1974; McArthur and Sengupta 1982). Diagnosis of the disease is based on clinical findings, presence of spores with characteristic morphology and size specific for each *Thelohanellus* species, and pathohistological structure of plasmodia. Also, a very reliable method of diagnostics is the molecular characterization of spores especially for the determination of new or similar species.

Moshu and Molnar (1997) described for the first time *Thelohanellus* infection of scales in 2-4 years old European wild carp (*Cyprinus carpio carpio*) collected

from water reservoirs of Moldova. Ćirković *et al.* (2009), Milošević (2009) and Novakov (2013) have detected and described *Thelohanellus nikolskii* infection of the scales in common carp (*Cyprinus carpio*) from Serbian fish farms. Changes are visible in the form of cysts on the scales reaching up to 3 mm in diameter.

The goal of this paper is to present and compare two different forms and clinical manifestations of diseases caused by *Thelohanellus nikolskii*.

MATERIAL AND METHODS

The investigation was conducted from 2008 to 2012 on 18 fish farms in Serbia (Bač, Svilojevo, Kolut, Sombor, Srpski Miletić, Ruski Krstur, Susek, Despotovo, Bečej, Žabalj, Lukino Selo, Mošorin, Sečanj, Banatski Dvor, Novi Itebej, Jazovo, Novi Kneževac, Kanjiža) and 4 fish farms in Bosnia and Hercegovina, Republic of Srpska (Prijedor, Prnjavor, Bardača, Brod). Fish farms were chosen taking into account to cover the area of supply of all rivers that are recipient of most carp ponds in Serbia.

The subject of the investigation was 1 to 4 year-old pond-cultured common carp (*Cyprinus carpio*). Sampling was conducted between April and October with intervals of 7-14 days. During each visit to the pond, clinical examinations was conducted on hundreds of fish. For laboratory examination, complete parasitological dissection was performed on 21 specimens of common carp. Fish were transported to the laboratory in plastic bags with water and oxygen. Clinical changes of the carps were photographed in the laboratory.

Light microscopy examinations of fresh smears from different organs were conducted during dissection by compression between 2 slides. The location of the cysts was recorded. Measuring of fresh spores and capsule length and width was performed by using software program Cell B.

Pathohistological examination was conducted by standard techniques: following fixation of the tissue samples, sliced to 5- μ m-thick sections, mounted and stained in haematoxylin and eosine. Micrographs were taken with Olympus BX51 microscope, using Color View III digital camera.

Artificial infestation of carp fingerlings for purposes of species differentiation and clinical monitoring was done in a fish pond in Mošorin which was only a few years old and uses well water free of parasites and intermediate hosts.

The influence of certain technological processes such as drying, freezing, mechanical processing of soil and disinfection with lime on the occurrence of thelohanellosis was monitored, also.

RESULTS

Thelohanellosis was diagnosed in all 22 investigated fish farms. The form of the disease which occurs on the fins was present in 60 days old carp fingerlings. Cysts present on fins were round and 1-2 mm in diameter. The prevalence of

infection ranged from 3-30%, and the intensity was 2 to 84 cysts per fish (Table 1). The disease was most intensely manifested in late July through August, while during the rest of the growing season was recorded sporadically. During the whole season malformations and defects of the fins were detected in carp fingerlings. Individuals who had a loss of tail fin substance became the prey of fish eating birds. Cysts were present in all fins (Figure 1). The majority of cysts were present on the tail fin, then on the dorsal, pectoral, abdominal and anal fins (Figures 1, 2).

Cysts on fins in two-year old carp fingerlings were present sporadically in few fish throughout the whole investigation, while in three-year old carps and older categories cysts were not observed at all.

In 2008, the cysts were recorded on scales in Susek, Žabalj, Jazovo and Bač fish ponds, while in the period 2009-2012 changes on the scales were present in all investigated farms. Plasmodia on the scales were present from the beginning of April until the end of May, while characteristic scale changes were not observed during the rest of the year. The prevalence of infection ranged from 2 to 75 %,

Table 1. Prevalence and intensity of infection of fins on investigated fish farms

Fish farm	Prevalence (%)	Intensity (number of cysts per fish)
Bač	6	10-55
Svilujevo	8	20-60
Kolut	4	7-23
Sombor	5	24-48
Srpski Miletić	15	6-51
Ruski Krstur	5	4-32
Susek	30	20-84
Despotovo	14	15-61
Bečej	6	12-38
Žabalj	25	10-64
Lukino Selo	10	6-61
Mošorin	8	2-36
Sečanj	5	4-28
Banatski Dvor	7	2-56
Novi Itebej	25	10-65
Jazvo	20	10-70
Novi Kneževac	6	6-28
Kanjiža	7	5-54
Prijedor	4	3-37
Prnjavor	6	4-35
Bardača	8	5-48
Brod	3	2-43

Table 2. The results of measurements of spores isolated from fins and scales.

Localization of spores	Spore length (µm)	Spore width (µm)	Capsule length (µm)	Capsule width (µm)
Scales	17.7 (13.5-20.3)	10.9 (10-12.1)	7.1 (6.1-8.7)	6.2 (5,8,6,6)
Fins	17.2 (13.4-20.6)	10.8 (9.2-13.3)	6.5 (5.8-7.6)	5.9 (5.2-6.8)



Figure 1. Cysts present on all fins of one-year old common carp.



Figure 2. Mature cysts on pectoral fins of one-year old carp fingerling.



Figure 3. The appearance of cysts present on scales of two-year old carp fingerlings.

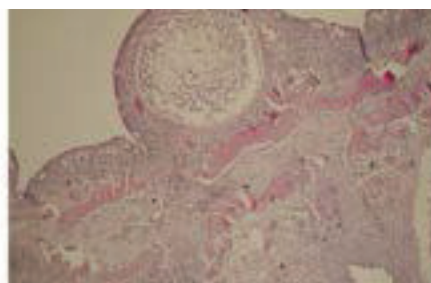


Figure 4. Cross section of one-year old carp finrays with present plasmodium. H&E, x200

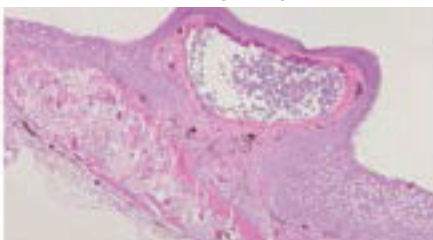


Figure 5. Cross section of scale with present plasmodium of one-year old carp. H&E, x200



Figure 6. Spores isolated from plasmodia of fins.

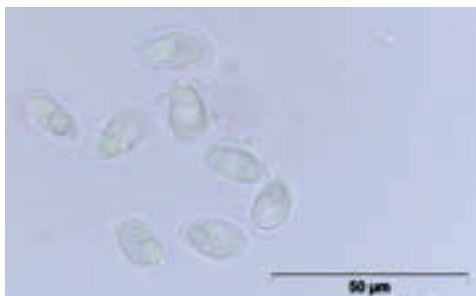


Figure 7. Spores isolated from plasmodia present on scales.

while its intensity was 2 -206 cysts per fish. Cyst were round and 1-3 mm in size and located on the outer margins of the scales (Figure 3). The form of the disease present on the scales was described in two-year-old fingerlings that were the most often infected, but also sporadically in three-year and four-year old common carps. Also sporadically, cysts were detected on the skin of four-year-old spawn carp. Cysts were not observed in one-year-old fry at all.

Artificial infestation of carp fingerlings was done in a fish pond in Mošorin. After entering a few individuals who had cysts on the scales, during the next vegetation period there were two forms of the disease, separately and at different times.

Histological structure of the fins is similar to the structure of the scales. The fin rays are built up from calcified collagen. The structure of the scales inside the dermis also consists of plates of calcified collagen. Cells surrounding the calcified collagen excrete collagen to the outer surface. Externally the dermis is covered by multilayered epidermis overlying a *membrana basialis*. The epithelium is rich in goblet cells which can be seen on histological sections.

After pathohistological examination it can be seen that in both forms of the disease the structure is almost the same (Figures 4, 5). Plasmodia on the fins and scales develops on fin rays and scale surface inside a calcified collagenic capsule (Figures 4, 5). Cartilaginous parts around the plasmodia break into pieces and calcified islets appear inside the connective tissue around the cyst (Figures 4, 5). The wall of the cysts around the plasmodium contain elongated cyst-forming cells. The central parts of plasmodia are filled with spores, while in the periphery vegetative developmental stages and pansporoblasts are present (Figures 4, 5). Deposits of melanin can be seen around the plasmodia. It is a special and unusual phenomenon of tissue reaction. Great plasmodia can be colored to black due to melanin or contain extracellular or intracellular deposits of melanocytes (Dycova, 1984).

For determination or description are using fresh spores, in exceptional cases spores fixed in 10% formalin can be used. The determination of the spores was done using light microscopy according to the methods determined by Shulman

(1984) and Lom and Arthur (1988). The shape and size of the spores are very important for the final determination.

Fresh spores were isolated from plasmodia of fins and scales and were compared to each other. In both cases isolated spores were oval, with one large polar capsule and sporoplasm (Figures 6, 7). Measurement values of fresh spores and polar capsules length and width are shown in Table 2.

DISCUSSION

The shape and size of *Thelohanellus nikolskii* spores from the plasmodia on the fins correspond to spores from scales, only the spores and polar capsules from the scales are slightly longer and wider. The shape and size of spores from fins and scales, also correspond to measurements of spores from fins given by Molnar (1982), Trombitsky *et al.* (1983), Ćirković (1986) and Dycova and Lom (1988).

By analyzing the pathohistological structure of the plasmodia from the fins and scales an almost identical structure, as described by Moshu and Molnar (1997) can be seen. By molecular characterisation of the parasite from the scales was confirmed that the etiological cause was *Thelohanellus nikolskii* Achmerov, 1955 is the same for both forms of the disease which occurs on the scales and on the fins (Novakov, 2013).

Also, results of artificial infestation indicate the presence of the same agent, but in two different clinical forms of the disease.

In one year-old carp fingerlings the location of the cysts are the fin rays, while in two years old and older carps the cysts are located on the scales. According to Moshu and Molnar (1997) in older fish due to advanced calcification of fin rays the cartilage is less suitable for plasmodium formation than are the scales.

The changes on the scales occur in April and May, and on the fins during July and August. Considering that, cysts on the fins which are present only in one-year old carp fingerlings can not mature before, rather is in the timing of the scales changes development, because in Serbia larvae are stocked during May and a certain amount of time is required in order to become visible.

Literature data reported by Janey (1979), Janey and Molnar (1981), Molnar and Kovach-Gayer (1981-1982), Molnar (1982), Ćirković (1986) and Ćirković *et al.* (1997), concluded that the prevalence and intensity of thelohanellosis on the fins is smaller than in the period of the mid-eighties. It is possible that this was affected by the application of freezing and drying of fish pond facilities for growing a fingerlings, as well as changes in technology which have occurred at the time. The prevalence and intensity of infection were most on fish farms where facilities for breeding are not strictly defined, and were rarely dried, frozen and mechanically cleaned, thus allowing intermediate hosts to be present during the next growing season. Also, the prevalence and intensity of infection were higher in facilities where stocking densities were higher, and therefore immunity and conditions of fish were weaker. Wasting due to thelohanellosis on the fins can be significant

due to loss of the fins. When the disease is present on the scales losses are not direct as after the cysts fall off only lesions and discolorations are evident.

The significant moment for thelohanellosis spreading is movement of fry between fish ponds where this disease was not taken into account. The second important factor is hydrographical connection between fish ponds, where water circulation enables transmission. Given the fact that the distance between fish ponds is small, fish eating birds are also an important factor in disease spreading.

During the experiment for the prevention and treatment of myxosporean Ćirković (1986) found that only nitrofurans are effective in the treatment of sphaerosporidiosis but not in the case of thelohanellosis. It was reported that the only efficacious chemotherapeutic drug against myxosporean diseases is fumagilin (Hedrick *et al.*, 1988; Székely *et al.*, 1988; Wishovsky *et al.*, 1990; Yokoyama *et al.*, 1990; El-Matbouli and Hoffmann 1991; Sitja-Bobadilla and Alvarez-Pellitero 1992; Rhee *et al.*, 1993; Yokoyama *et al.*, 1999). However, fumagilin could not be used for the treatment of myxosporean diseases due to unresolved problem with toxic residues in tissues and the withdrawal time of the drug. So the control of thelohanellosis is still based on compliance with basic sanitary-prophylactic measures such as drying of objects, freezing, mechanical cleaning and disinfection with lime.

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RAZLIČITE MANIFESTACIJE OBOLENJA IZAZVANOG THELOHANELLUS NIKOLSKII KOD ŠARANA (CYPRINUS CARPIO)

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SADRŽAJ

Telohaneloza je obolenje koje se javlja kod svih kategorija šarana. Uročnik je protozoa *Thelohanellus nikolskii* Achmerov, 1955. Manifestuje se u dva oblika. Prvi se javlja kao telohaneloza na perajima kod jednogodišnjih mladunaca šarana a drugi kao telohaneloza krljušti kod dvogodišnjih, trogodišnjih i višegodišnjih kategorija ribnjačkog šarana (*Cyprinus carpio*). Istraživanjima koja su trajala u periodu od 2008.-2012. godine obuhvaćeno je 22 ribnjaka od čega se 18 nalaze u Srbiji a 4 u BiH, Republika Srpska. Utvrđeno je da su oba oblika obolenja bila prisutna na svim ispitivanim ribnjacima. Kod telohaneloze na perajima, prevalenca se kretala od 3-30% a intenzitet infekcije od 2-84 cisti po jedinki, dok su te vrednosti kod telohaneloze na krljuštima bile veće. Promene na krljuštima bile su prisutne tokom aprila i maja a na perajima tokom jula i avgusta.

Utvrđivanjem morfoloških karakteristika spora iz cisti sa peraja i krljušti, kao i prilikom determinacije patohistoloških karakteristika plazmodijuma nisu uočene značajne razlike između njih. Takođe je urađen i ogled veštačkog zaražavanja mladunaca šarana radi diferencijacije vrste.

Pošto ne postoji ni jedno adekvatno terapeutsko sredstvo kontrola telohaneloze i dalje se bazira na pridržavanju osnovnih sanitarno profilaktičkih mera kao što su isušivanje objekata, izmrzavanje, mehanička obrada tla i dezinfekcija krečom.