OCCURRENCE OF MYXOBOLUS ENCEPHALICUS (MUSLOW 1911) IN POLAND: POSSIBLE RELATIONSHIP BETWEEN THE PARASITE INFECTION AND CLINICAL SYMPTOMS IN COMMON CARP (CYPRINUS CARPIO)

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Abstract

The common carp (Cyprinus carpio) originating from 18 farms, where unexplained cases of disease symptoms and fish mortality appeared, were examined for the presence of Myxobolus encephalicus. Various stages of parasite development and host macrophage reaction were demonstrated in fresh compressed preparations and in histological sections. M. encephalicus appeared to be present in all 18 carp farms. Possible relationship between the parasite presence in carp brain and carp mortality are discussed.

Key words: carp, Myxobolus encephalicus, parasitoses, symptoms.

Myxobolus encephalicus (Muslow 1911) infects carp (Cyprinus carpio) brain and spinal cord. The parasite belongs to phylum Myxozoa, class Myxosporea (Buetschli 1881). The presence of M. encephalicus in carp brains was reported in Hungary (4), Czechoslovakia (7) and Russia (11). In Poland, in 1970, 2 cases of Myxobolus sp. possible Myxobolus encephalicus infection in carp fingerling were described by Hlond (6) and Witala (14). Since that time no reports concerning M. encephalicus could be found until 2002 when Antychowicz (1, 2, 3) described several cases of the parasite infection in common carp and Matras (9) found the parasite in koi carp.

Myxobolus encephalicus life cycle is unknown and the parasite pathogenicity for carp as well as the effect of its infection on fish organism is still not clear. As the hitherto unexplained nervous symptoms in common carp accompanied with mortality were reported quite often for the last years, the presented investigations have been performed.

Material and Methods

The carp from 18 farms (15 farms located in Lublin district), 5 from each facility, were examined parasitologically with special emphasis for the presence of M. encephalicus plasmodia and spores in the brain.

The investigations lasted three years, and were performed in 2002, 2003 and 2004. During this period, 90 carp brains were isolated and compressed preparations as well as occasionally histological sections were made. The whole brains were examined each time by means of light microscopy and photographic documentation was collected. The virological examination was also performed using the technique described in EU directive (5) and OIE Manual (10), with special emphasis on SVC virus detection. The clinical and histological examinations of the fish were made for the presence of lesions induced by bacteria in the integument and internal organs, and also general parasitological examinations were performed.

The histological sections were prepared using the routine methods. In short; carp brains were fixed in 10% buffered formalin solution, washed, dehydrated in serial of increasing alcohol concentrations, cleared in xylene, and then embedded in paraffin. Paraffin blocks were cut at 5 µm and affixed to glass slides. Finally, the sections were stained with haematoxylin and eosin and examined under light microscope.

Results

In the carp originating from 18 farms, where unexplained clinical symptoms and mortality were reported, M. encephalicus was found (Table 1). In some cases the presence of the parasite was showed in all 5 fish which represented particular farm.
Table 1
Results of the investigations for the presence of *Myxobolus encephalicus* (Buetschli 1881) in carp from 18 Polish farms in 2002-2004

<table>
<thead>
<tr>
<th>No</th>
<th>date</th>
<th>presence of early stages of sporogeny</th>
<th>presence of plasmodia and spores</th>
<th>presence of macrophages</th>
<th>symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.04.2002</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>mortality</td>
</tr>
<tr>
<td>2</td>
<td>5.10.2002</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>mortality, sunken eyes</td>
</tr>
<tr>
<td>3</td>
<td>23.10.2002</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>mortality, scratching behaviour</td>
</tr>
<tr>
<td>4</td>
<td>14.02.2002</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>mortality, scratching behaviour</td>
</tr>
<tr>
<td>5</td>
<td>4.03.2003</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>grouping near the dikes, scratching behaviour</td>
</tr>
<tr>
<td>6</td>
<td>8.03.2003</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>mortality, lying on the bottom, sunken eyes</td>
</tr>
<tr>
<td>7</td>
<td>22.05.2003</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>mortality, lying on the bottom, sunken eyes</td>
</tr>
<tr>
<td>8</td>
<td>25.05.2003</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>mortality, lying on the bottom, sunken eyes</td>
</tr>
<tr>
<td>9</td>
<td>10.07.2003</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>mortality, lying on the bottom, sunken eyes</td>
</tr>
<tr>
<td>10</td>
<td>17.07.2003</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>mortality, grouping in the reeds</td>
</tr>
<tr>
<td>11</td>
<td>17.10.2003</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>mortality</td>
</tr>
<tr>
<td>12</td>
<td>19.11.2003</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>grouping near the dikes</td>
</tr>
<tr>
<td>13</td>
<td>16.01.2004</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>scratches in the skin</td>
</tr>
<tr>
<td>14</td>
<td>30.01.2004</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>lack of winter hibernation</td>
</tr>
<tr>
<td>15</td>
<td>14.02.2004</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>lack of winter hibernation</td>
</tr>
<tr>
<td>16</td>
<td>18.04.2004</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>no symptoms</td>
</tr>
<tr>
<td>17</td>
<td>15.08.2004</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>apathy, grouping, mortality</td>
</tr>
<tr>
<td>18</td>
<td>20.09.2004</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>emaciation, sunken eyes</td>
</tr>
</tbody>
</table>

Fig. 1a. Early sporogenic forms in brain vessel, fresh preparation, bar 20µm.
Fig. 1b. Early sporogenic forms, bar 20µm.
Fig. 2. Ripe spores, equipped with pair polar capsules, fresh preparation, bar ▬▬ 20µm.

Fig. 3. Elongated polysporic plasmodia, fresh preparation, bar ▬ 20µm.

Fig. 4. Large group of spores, fresh preparation, bar ▬ 20µm.

Fig. 5. Macrophage proliferations among congregate spores, fresh preparations, bar ▬▬ 20µm.

Fig. 6. Spores destructed by macrophages, fresh preparation, bar ▬▬ 20µm.

Fig. 7. Densely congregated, destructed and encapsulated spores, fresh preparation, bar ▬ 20µm.

Fig. 8. Encapsulated spores in meninges, histological preparation H.E, bar ▬▬ 20µm.

Fig. 9. Spores in the vicinity of brain blood vessels, histological preparation H.E, bar ▬ 40µm.
According to the fish farmers who have delivered the fish for examination, the following symptoms in carp had appeared: scratching against the bottom, grouping with the signs of apathy, lack of hibernation in winter, emaciation, and sunken eyes.

In laboratory, besides emaciation and sunken eyes which appeared in some fish, we observed in some fish also delicate scratching marks on the skin.

In carp brains the following development stages of the parasite were found: possibly the early sporogenic forms in brain vessel, 8–13 µm of size (Figs 1a, 1b), ripe spores equipped with pair of polar capsules, 8–11 µm of size (Fig. 2), elongated polyosomal plasmodia of variable size, some up to 400 µm in length (Fig. 3). The spores were scattered in the brain tissue singly, in little groups (3-8 spores) or in large groups (Fig. 4).

In some fish macrophage proliferations among congregated spores were observed (Fig. 5). Possibly the end effect of macrophage activity was spore destruction which was shown on the Fig. 6. Sporadically phagocytized and encapsulated (possibly with host tissue) densely congregated spores were noted (Fig. 7).

The histological examination showed that M. encephalicus plasmodia could be found in the all parts of the brain and all brain strata, among others in the meninges (Fig 8), but in the majority of cases they were found in the vicinity of brain blood vessels (Fig 9). The examinations of fresh compressed preparations made from the fish brain tissue demonstrated that some of the early sporogenic stages of the parasite showed periodic rotating movements. We found that M. encephalicus can affect carp of varied size and age, including small fingerlings (4-5 cm) and three years old fish, more than 35 cm in length. The number of M. encephalicus plasmodia and spores even in fish originating from the same farm usually differ greatly. In some cases the parasites were very numerous and reached up to 60 plasmodia or hundreds of thousands spores per one little brain which measures 6-8 mm in length.

Usually no other than M. encephalicus parasites were found in fish with above described symptoms but if any other parasites were present, their numbers were insignificant.

No viruses were found, at least no one which would be able to grow in the cell lines used in our laboratory i.e. EPC, FHM, BF-2 and RTG-2. The examined fish did not show symptoms or lesions such as ulcers, petechiae, integument inflammation, gill necrosis suggesting the presence of bacterial infection. Besides, neither bacterial microcolonies nor histopathological lesions in organs located in the body cavity were detected.

Discussion

Lom and Dykova (7, 8) mentioned, that M. encephalicus is widespread in Europe, nevertheless, there is scarcity of information concerning the parasite.

Sporadic cases of M. encephalicus infection in common carp were described in Hungary, Russia and Poland. Since 1970 the presence of M. encephalicus was not reported in Poland, though there were noted many hitherto unexplained cases of carp mortality which were accompanied by nervous symptoms. In 18 such cases various developmental stages of the parasite were detected in carp brain. Considering that there were no other obvious etiological (infec tious or non-infectious) agents discovered, M. encephalicus appeared to be the main suspect. In carp reared with traditional system in large ponds, the fish diseases are usually the results of a synergistic complex of disease and a particular etiological agent, which is usually difficult to establish.

On the basis of our observations some phenomena which often appear during M. encephalicus infection could be considered as eventual evidence of the parasite pathogenicity for fish.

Our investigations have confirmed that M. encephalicus plasmodia could block the brain vessels and disrupt them but also showed that the brain vessels could be clotted with the parasite early sporogenic stages. It seems very probable that disturbances in blood flow and sometimes also the presence of local granulomatous inflammation in the brain tissue could irritate the carp central nervous system strongly enough to cause the appearance of nervous symptoms in the fish. These factors could probably prevent proper carp hibernation during winter. If carp do not hibernate they swim constantly. In a densely populated winter pond where feeding is not possible this situation results in the depletion of organism energy resources and eventually in fish mortality. It is worth noting that the number of parasites in a single little carp brain is sometimes very high and can reach up to 60 plasmodia or hundreds of thousands spores. In some cases, besides plasmodia and spores also early presporogenic stages were present. These stages of the parasite development could cause blood vessel clotting and dilatation.

Lom and Dykova (7, 8) expressed the opinion that when fishes infected with M. encephalicus showed emaciation and sunken eyes symptoms, the prognosis for fish survival is poor. On the other hand, the authors wrote that there was no reliable evidence that M. encephalicus could cause great losses in carp populations. In 18 cases of M. encephalicus infection which we have investigated it was impossible to exclude completely the negative effect of the environment on fish mortality but also the role of M. encephalicus as the factor causing the above described symptoms should not be underestimated.

According to Plehm (13), the heavy infection of carp fry and fingerlings with M. encephalicus results in the fish locomotory disorders demonstrated with loss of balance or swimming in circles and sometimes hanging motionless in the water head down. Hlond (6) and Witala (14) suggested that there may be the relationship between the parasite presence in the carp brain and various nervous symptoms observed in the fish. This corresponds well with our observations (Table 1).
The further investigation of pathogenesis of *M. encephalicus* infections should be made to clarify the relationship between the parasite infection and carp losses. On the basis of reference data and our own investigations accomplished during 3 years it could be concluded that *M. encephalicus* is actually the most dangerous carp parasite in Poland comparable only to *Ichtyophthirius multifiliis*.

References

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