

The occurrence of *Sphaerospora renicola* K-stages in the choroidal rete mirabile of the common carp

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Abstract. It is known from earlier studies that the so-called K-protozoa, the late presporogonic stages of *Sphaerospora renicola*, a parasite causing renal sphaerosporosis in common carp (*Cyprinus carpio*), accumulate in the swimbladder wall and give rise to the disease called myxosporrean swimbladder inflammation. The present study indicates that in the case of intensive infection these parasites also occur in the rete mirabile of the choroid of the eye, causing haemorrhages and necrosis. The results suggest that the K-stages of *S. renicola* are located in the blood path just like the early presporogonic C-stages; however, as they get stuck in organs rich in capillaries, it is difficult to demonstrate them from the blood of the common carp.

Since the works of Molnár (1980), Kovács-Gayer et al. (1982), Körting (1982) and Csaba et al. (1984) led to the identification of parasites designated as K-protozoa (the letter K in K-protozoa comes from the initials of Kovács-Gayer and Körting, who were the first to detect this developmental stage), causing swimbladder inflammation in common carp, with the presporogonic developmental stages of the kidney parasite *Sphaerospora renicola* Dyková et Lom, 1982, a vast amount of knowledge has been accumulated on the complex developmental cycle of *Sphaerospora*-type parasites. It has become known that the early developmental stages described by Csaba (1976) and designated as C blood protozoa by Molnár (1980) and UBO by Lom et al. (1983) consistently appear in the blood of carp before renal sphaerosporosis develops. The works of Lom et al. (1985) have revealed that the blood stages can be also demonstrated in the blood of other *Sphaerospora*-infected cyprinid fishes. Moreover, in the blood of these cyprinids Baska and Molnár (1988) found circulating forms morphologically resembling the K-stages of the common carp. In common carp, the K-stages were earlier detected first of all in the swimbladder wall (Kovács-Gayer et al. 1982, Körting 1982, Csaba et al. 1984, Landsberg 1986, Odening 1987). The possible occurrence of K-stages in other locations has only been suggested by Körting (1983), Körting et al. (1984) and Ter Höfte et al. (1984), who found the developmental stages in the integument of the head, in the orbit as well as in hepatic granules of the common carp. Recently, Lom et al. (1991) have found *Sphaerospora*

stages resembling K-protozoa in the rete mirabile of non-cyprinid fish (*Gasterosteus aculeatus*), though they failed to clearly identify these forms with the developmental stages of *S. elegans* found in the kidney.

The present paper furnishes data to support the view that *S. renicola* K-stages, earlier considered to be typical swimbladder parasites, are blood stages just like C-parasites; however, they are frequently caught in the capillaries. These stages can also produce lesions in organs other than the swimbladder, and are directly responsible for the exophthalmos regarded as a sign of swimbladder inflammation, as well as for the intraorbital haemorrhages and tissue necrosis.

MATERIALS AND METHODS

Thirty-four 2.5-3-month-old common carp fry, showing clinical signs of swimbladder inflammation, were examined. The fish measured 4-8 cm in length and came from farm ponds. After killing the fish, the swimbladder and the choroid of one eye were removed. From both organs impression smears were prepared and stained with Giemsa. The above organs of the same fish were examined also in the form of fresh preparations under a coverslip for infection by K-protozoa. Simultaneously, a small, pathologically altered portion of the swimbladder and the entire other eye were fixed in Bouin's solution. If one of the eyes or the swimbladder proved to be infected by K-protozoa when examined either in fresh state or as impression smears, the fixed organs were processed for histological examination. Paraffin-embedded, 4 µm thick sections stained with haematoxylin and eosin served as histological preparations. Photomicrographs were taken of these preparations using a Zeiss photomicroscope. A drop of blood, a piece of the kidney squashed under a cover-

slip, and a small portion of rete mirabile of the choroid removed free from pigment contamination, as well as that of the rete mirabile of the swimbladder were examined in a microscope as fresh preparations for the occurrence of other *Sphaerospora* stages.

RESULTS

All specimens of 4–8 cm long common carp fry showing the signs of swimbladder inflammation harboured sporogonic stages of *S. renicola* in the renal tubules. The C-blood stages were consistently demonstrable in fresh preparations made from the peripheral blood or choroid of these fish. At the same time, the K-stages could be found in impression smears made from the swimbladder only in the acute cases. K-protozoa were found in the swimbladder wall in 14 out of the 34 examined fish showing swimbladder lesions. *Sphaerospora* K-stages were detected in the orbit of 3 out of these 14 fish by an examination of smears stained with Giemsa. The K-stages were demonstrated in fresh preparations from two fish showing severe exophthalmos and intra-orbital haemorrhage, and these formations were easily observable also in histological sections made from the same fish.

In addition to cellular elements of host origin, numerous, mostly round or amorphous primary parasite cells 19–23 μm in diameter could be found in Giemsa-stained smears prepared from the choroid (Fig. 1). Depending on their developmental status, these formations contained 2–15 secondary cells. In specimens less deformed during smear preparation, inclusion-like secondary cells situated within the primary cell abounding in cytoplasm were clearly visible. The majority of secondary cells contained two tertiary cells each.

The K-stages could also be studied in fresh preparations of the choroid carefully squashed under a coverslip, if the choroid had been removed free from pigments of retinal origin. In such cases, the K-stages appeared as typically round, signet-ring shaped formations. Because of their pale colour and immobility, they could be distinguished from the surrounding cells only after the preparation had been sufficiently flattened. The mass of secondary cells present in an inclusion situated centrally in the primary cells was clearly visible also in these formations.

At low magnification, the histological preparations made from transverse sections of the choroid of the eye (Fig. 2), showed an extreme dilatation of the capillaries and the presence of extensive haemorrhages, which were striking. In preparations examined at a higher magnification (Fig. 3), besides the haemorrhagic choroid layer a zone containing disintegrated necrotic cells with signs

of degeneration by karyorrhexis could be distinguished. The two layers were demarcated by an anaemic area containing numerous K-protozoa (Fig. 4). The capillary structure of the choroid was indistinct in this area. In a certain part of the parasites, triple formations comprising two tertiary cells and a secondary cell could also be demonstrated histologically (Fig. 4, inset).

A marked size difference was found between *Sphaerospora* K-stages found in impression smears and those examined in histological sections. In impression smears, the diameter of the flattened parasites ranged between 19 and 23 μm ; at the same time, the formations that had become shrunken during histological processing were only 8–12 μm in diameter. Similarly, there was a size difference also between K-protozoa demonstrated in the eye and those found in the swimbladder. In histological sections, the diameter of parasites derived from the choroid was 8–12 μm , in contrast to formations occurring in the swimbladder, which were 12–15 μm in diameter. A difference was also found in the number of secondary cells situated within the primary cell. Primary cells occurring in the eye never contained more than 15 secondary cells, whereas those from the swimbladder occasionally had as much as 40 secondary cells.

DISCUSSION

Earlier data (Körtling 1983, Körtling et al. 1984, Lom et al. 1985, Baska and Molnár 1988) already indicated that *Sphaerospora* K-stages had no swimbladder specificity and were likely to occur throughout the body. However, to date, no data have become available on the tissue affinity of these formations. Although in a histopathological work Kovács–Gayer (1983) gave a detailed description of the occurrence of K-protozoa in different layers of the swimbladder, she failed to provide data on whether they occurred within the capillaries or in intracellular location. Even the electron microscopic examinations (Molnár 1988, Dyková et al. 1990) have failed to furnish data in this respect. The demonstration of these parasites from the blood stream of other cyprinid fishes (Baska and Molnár 1988) spoke in favour of intracapillary location. Although no absolute histological proof of the intracapillary location of K-stages could be obtained in this study, the occurrence of these parasites in organs rich in capillaries and the haemorrhages caused by them in those organs undoubtedly indicate that *Sphaerospora* K-stages develop within the blood path also in the common carp. The intensity of *Sphaerospora* infection is higher in common carp than in other fish (Molnár, unpublished); presumably this is why the parasites regularly get stuck

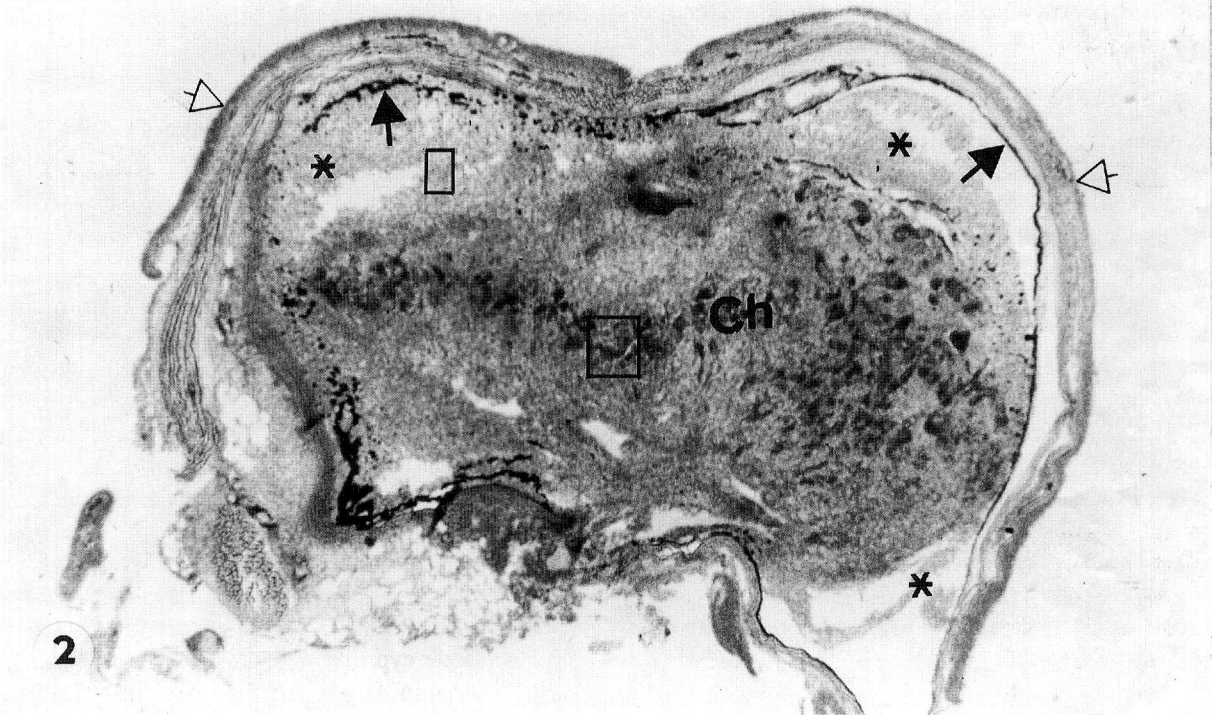
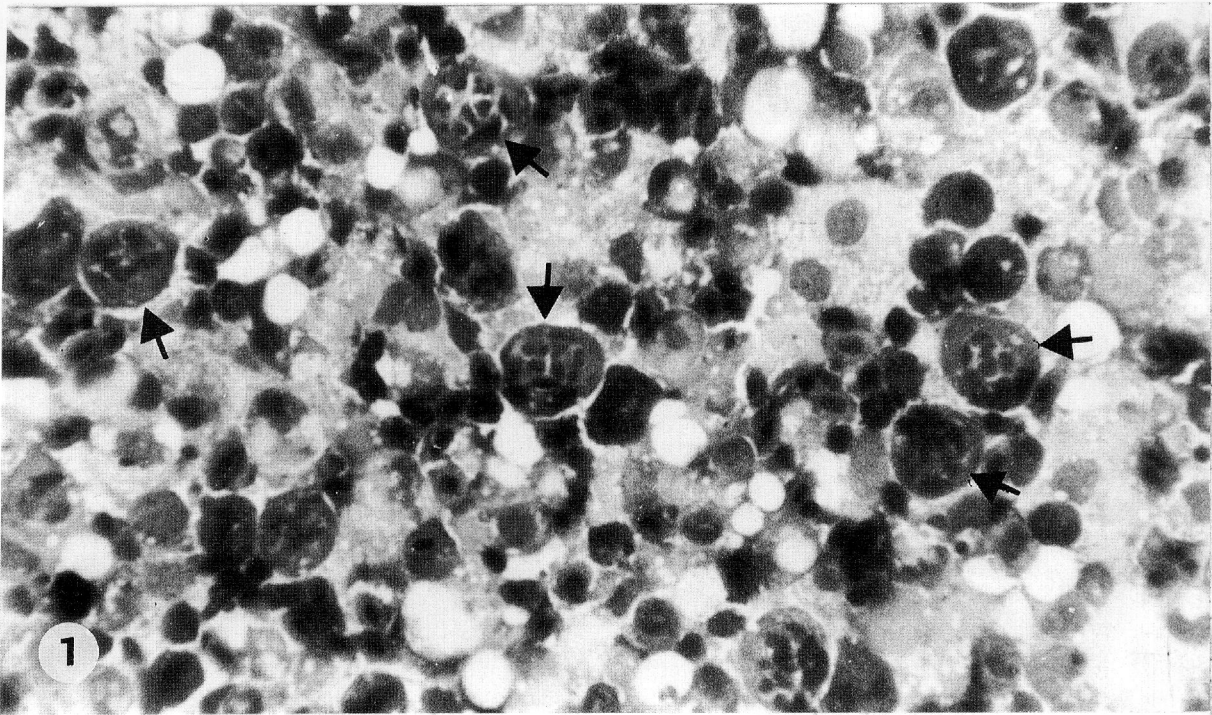


Fig. 1. K-protozoa (arrows) in an impression smear made from the choroid layer of the eye of common carp. Giemsa staining (x 400).
Fig. 2. Cross-section of the eye of common carp fry specimen in the posterior part of the eye bulb. Rete mirabile of the choroidea (Ch) is surrounded by the sclera (white arrows). A pigmented layer of the sclera (black arrows) encapsulates choroidea which contains dilated capillaries, haemorrhages and K-protozoa in the centre and degenerated cells (*) in the periphery. Boxes represent the areas enlarged in Figs. 3 and 4. Haematoxylin and eosin (x 40).

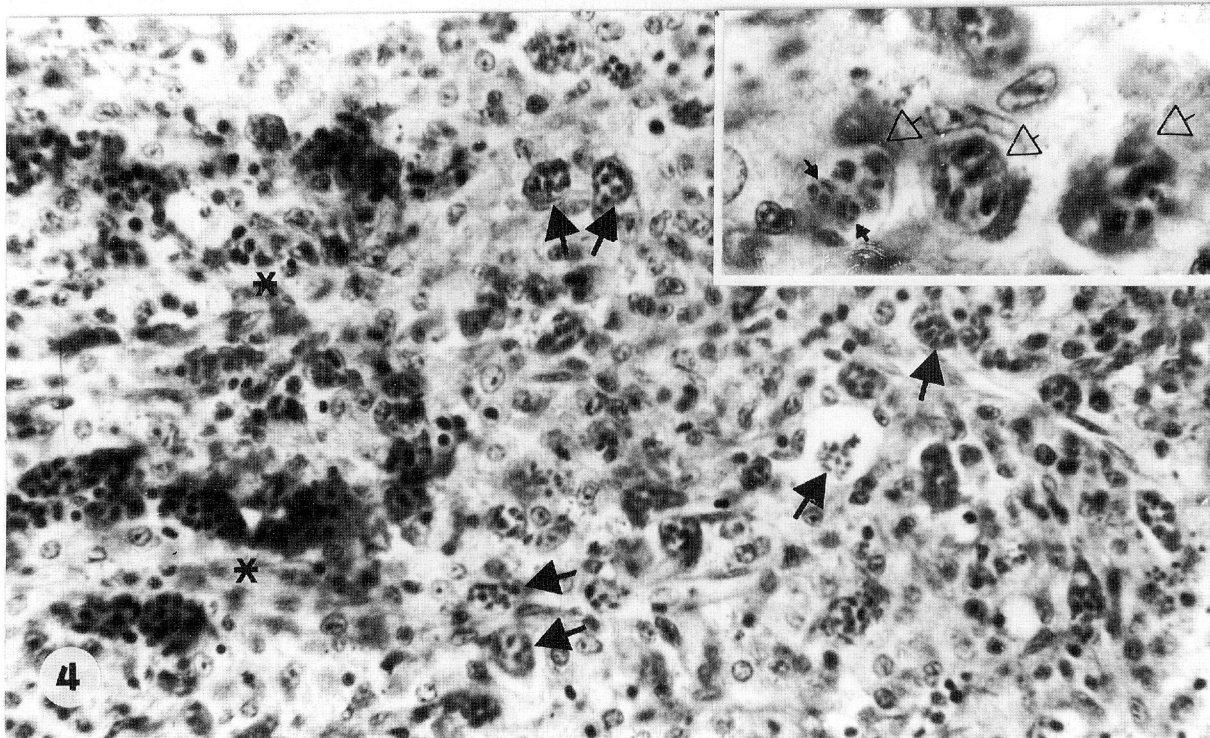
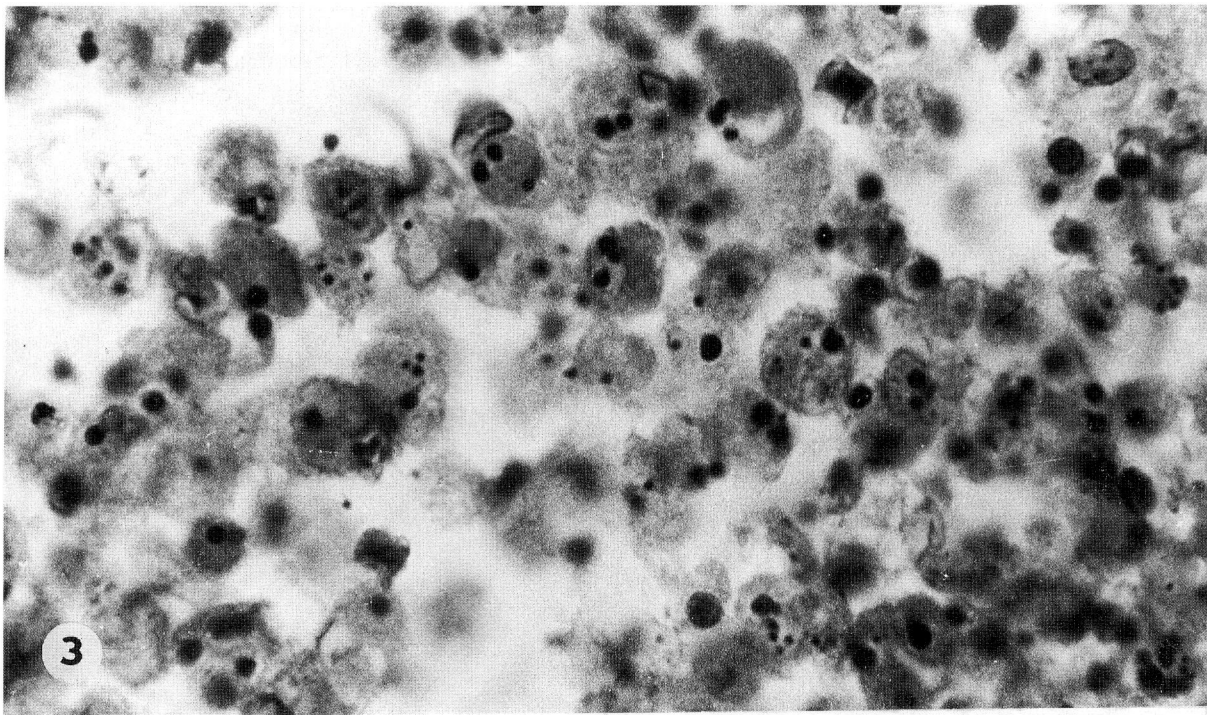


Fig. 3. Degeneration by kharyorhexis in the periphery of rete mirabile due to an infection with K-protozoa. Histological section. Haematoxylin and eosin (x 1,200). **Fig. 4.** K-protozoa in the eye of common carp fry. Numerous parasite specimens (arrows) are located in the rete mirabile beside dilated capillaries and haemorrhages (*). Histological section. Haematoxylin and eosin (x 500). Inset: K-protozoa (large arrows) containing triple formations (small arrows) in the eye of the common carp. Histological section. Haematoxylin and eosin (x 1,500).

in the rete mirabile in the common carp, accumulate there and give rise to pathological lesions in the organs involved. It cannot be ruled out, however, that the parasites get caught in the capillaries because of the biological characteristics of the rete mirabile in the common carp. Namely, the same parasites are regularly demonstrable from the blood of goldfish, a species closely related to the common carp (Székely, personal communication). Obstruction of the capillaries in a relatively circumscribed site results in disturbances of the blood flow, and obviously this accounts for the dilatation of capillaries and the small haemorrhages occurring both in the swimbladder and in the choroid of the eye. In the case of the swimbladder this process, accompanied by tissue swelling, haemorrhages and extravasation of blood elements and serum into the interstitial space, was designated as a swimbladder inflammation, though the inflammatory character of purely parasitic swimbladder inflammation had not been widely demonstrated (Kovács-Gayer 1983) and, based on earlier assumptions, secondary bacterial processes were held responsible for the majority of the tissue changes (Csaba et al. 1984). The congestion, haemorrhages and even necroses observed in the choroid in this study

clearly indicate that the systemic and local signs of swimbladder inflammation, a disease earlier thought to be of viral (Ahne 1973) or bacterial (Szakolczai 1967, Mattheis and Kulow 1967, Kocylowski et al. 1970) aetiology, can be attributed to the pathomechanical effect of parasites. It seems that in less intensive infection only the swimbladder becomes involved; however, in the case of massive infection, masses of K-protozoa also colonize the capillaries of the eye, giving rise to extensive necrotic lesions. These lesions unambiguously prove that – as has been pointed out by Körtling et al. (1984) – the exophthalmos and periorcular haemorrhages, described earlier as the accompanying signs of swimbladder inflammation, are a direct consequence of capillary obliteration caused by K-protozoa, rather than resulting from the oedema induced by bacterial invasion supervening on parasitic infection. The histological examinations prove that in the area before the capillary portion obliterated by K-protozoa, intracapillary congestion and haemorrhages occur, while the area beyond the blockade becomes anaemic. The choroidal necroses observed in severe infection by K-parasites are obviously a direct consequence of parasite-induced obliterations and haemorrhages as well as partial anaemia.

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