INTRODUCTION

The vertebrate kidney is a paired organ with a complicated structure of epithelial, connective tissue and endothelial elements (Harder 1975). The majority of the kidney, including the Bowman’s capsules, urinary channels and ureters serves an excretory function, while the head kidney and the interstitial part of the trunk-kidney is haematopoietic, with blood-forming and recycling functions or roles. Melano-macrophage centres imbedded within interstitial tissues play a major role in defence. The kidney has an endocrine function in the corpuscles of Stannius, suprarenal and interrenal cells.

Myxozoan parasites of fish are commonly found in the kidney, and it is becoming apparent that the specific pattern of development and final site of sporogenesis can vary among parasite species. Coelozoic development of different Sphaerospora spp. has been known for a long time (Thélohan 1892, Fujita 1912), but only Csaba et al. (1984) revealed that these sporogenic forms were preceded by blood-dwelling presporogonic stages. The change from epithelial to coelozoic stage in the development of Hoferellus cyprini was recognised by Plehn (1924). A Hoferellus-like development was also described by Molnár (1988) for Myxobilatus legeri (Cépede, 1905). Similarly, Lom et al. (1989) revealed that large xenoma-forming plasmodia of Myxidium giardi Cépede, 1906 were released from the renal corpuscles into the coelozoic space of renal tubules as small trophozoites. Plehn (1925) first mentioned the intercellular development of a myxozoan in the kidney, with a description of the development of Sphaerospora tincae in the head kidney of the
tench. This isolated intercellular location among the haemopoetic cells was confirmed with electron microscopy by Lom et al. (1985a). A specific combination of intercellular and coelozoic developments characterise Tetracapsuloides bryosalmonae (Canning, Curry, Feist, Longshaw & Okamura, 1999), the causative agent of proliferative kidney disease. Extrasporogonic stages of this myxozoan develop intercellularly among the cells of the renal interstitium; the spores, however, are formed in the renal tubules (Kent et al. 2000, Feist et al. 2001). Little is known about how myxozoans form large plasmodia in the kidney. Although it has been known for some time (Dyková et al. 1987) that plasmodia of Myxidium rhodei Léger, 1905 can form large plasmodia inside the renal corpuscles of cyprinids, little is known about the validity of the great number of Myxobolus spp. listed from the kidney by Shulman (1966). Dispersed spores and large groups of Myxobolus spores are commonly found in the renal parenchyma, but without observation of the developing stages it is difficult to determine whether the spores developed at the site or were carried and accumulated there by the host macrophages. Molnár & Kovács-Gayer (1985) observed that, following a heavy infection by the muscle-dwelling Myxobolus cyprini Doflein, 1898, large groups of spores were accumulated in melano-macrophage centres of the kidney. Molnár (1994) suggested that myxozoans have certain species, organ and tissue specificity (of which tissue specificity is particularly important).

Here, I characterise the types of establishment and development of myxozoan plasmodia in the kidney, on the basis of long-term experience in the study of myxozoans infecting Hungarian fishes.

**MATERIALS AND METHODS**

During >40 yr of research in the field of fish parasitology, I frequently found myxozoan infections in the kidneys, which through careful histological study could be traced to specific sites inside this organ. Records involving infections of the kidney with myxozoan parasites were evaluated, and the site selection of different species was characterised. Histological sections were prepared of kidneys of various fish species infected with different developmental stages of species of Myxobolus, Myxidium, Hoferellus, Myxobilatus and Sphaerospora. Uninfected fish were also examined as controls. Samples evaluated were collected from 7 fish species, including sterlet Acipenser ruthenus, common carp Cyprinus carpio, white bream Blicca bjoerkna, roach Rutilus rutilus, rudd Scardinius erythrophthalmus, pike Esox lucius, rainbow trout Oncorhynchus mykiss and eel Anguilla anguilla. When pieces of kidney tissue compressed between 2 glass plates showed myxozoan infection under a compound microscope, differently sized parts of the infected organ were fixed in Bouin’s solution for 4 h. Fixed samples were washed several times in 80% ethanol, embedded, cut into 4 to 8 µm thick sections, and stained with haematoxylin and eosin using the Farkas-Mallory method (Kiszely & Barka 1958). Photomicrographs were taken with video equipment attached to an Olympus microscope, and images were digitalized with the software package Imago (Székely 1997).

**RESULTS**

In most cases, the occurrence or development of these parasites occurred in a specific part or tissue of the kidneys. Five major sites of infection were identified.

Coelozoic development in the renal tubules

In freshwater fishes, species of Sphaerospora are common inhabitants of the urinary channels. Of the nearly 50 nominal species in the genus, S. renicola Dyková & Lom, 1992 is the best known. Sporogonic stages of most Sphaerospora spp. inhabit the lumen of the urinary ducts.

In most cases, developing pseudoplasmodia and spores were found simultaneously in the renal tubules. Pseudoplasmodia were located in the anterior segments of the proximal tubules, which differ from the posterior segments by the presence of columnar epithelium (Fig. 1), while spores were mostly located in the posterior segments of the distal tubules that were lined by a cuboidal epithelium. Pseudoplasmodia, which correspond to the pansporoblasts of large plasmodia, comprised a nucleus of the mother cell and 2 sporoblasts. Inside each sporoblast, 6 nuclei developed, forming a single spore. Thus, at an advanced stage of sporulation the pseudoplasmodium might be composed of at most 13 cells (Fig. 2). Pseudoplasmodia and mature spores may tightly fill the lumen of the ducts. As a rule, developing pseudoplasmodia were attached to the urothelium of the ducts, but mature pseudoplasmodia with a decaying mother cell and 2 spores were usually found free in the lumen (Fig. 2). Both developing and mature spores were typically found in pairs. Presporogonic stages developing in the blood entered the tubules through the glomeruli of the Bowman’s capsules. Sphaerospora-like parasites had a relatively short development time. The coelozoic stages in fingerlings were common during the summer, whilst in brood fishes they were frequently found during the spawning period.
Molnár: Site preference of myxozoans in fish kidneys

Development in the renal corpuscles

Development of vegetative stages of several species of Myxidium and Chloromyxum often takes place within the renal corpuscles inside the Bowman’s capsule. Early developmental stages of Myxidium lieberkuehni Bütschli, 1882, a parasite of pike Esox lucius, were found in the renal corpuscles (Fig. 3). Growing plasmodia of the parasite deformed the structure of the Bowman’s capsule (Fig. 4). Plasmodia containing primary, secondary and tertiary cells entered the tubules and formed spores during this coelozoic stage. Very often, enormously enlarged plasmodia became encapsulated. These plasmodia, referred to previously as a separate species, Nephrocystidium pickii, contained the rest of the glomerular capillary and a large nucleus (Fig. 5), and were surrounded by a layer of host connective tissue. A similar development characterises Myxidium rhodei Léger, 1905, a parasite of cyprinids. In M. rhodei, however, the whole development very often took place inside the Bowman’s capsule, where the mature spores were also found (Fig. 6).

Figs. 1 to 4. Fig. 1. Pseudoplasmodia (p) of Sphaerospora renicola in the lumen of the proximal segment of the renal tubule of common carp. This segment of the tubules is lined by columnar epithelial cells (e) with a prominent brush border. H&E staining. Scale bar = 10 µm. Fig. 2. Infection of rudd renal tubule by Sphaerospora sp. Pseudoplasmodia (arrowheads) containing at most 13 nuclei are loosely attached to the epithelium; aged pseudoplasmodia harbouring 2 spores (arrows) are free in the lumen. Urothelium in this part of the tubules is made up of cuboidal epithelial cells (e). H&E staining. Scale bar = 10 µm. Fig. 3. Cross-sectioned renal tubules and a glomerulus (g) in pike kidney. Trophozoites of Myxidium lieberkuehni are indicated (arrows) inside the Bowman’s capsule and in the tubules. H&E staining. Scale bar = 40 µm. Fig. 4. Bowman’s capsule in pike kidney harbouring large plasmodia (p) of M. lieberkuehni and surrounding the rest of the glomerulus (g). H&E staining. Scale bar = 40 µm.
Intraepithelial development in the renal tubules

This type of development is characteristic of species of *Myxobilatus* and *Hoferellus*. The vegetative stage of this development occurred in the epithelial cells of the renal tubules (Figs. 7 & 8) where primary, secondary, tertiary and quaternary stages of the parasites might be formed intraepithelially. The stages in the initial part of sporulation either actively emerged from the infected host cells and entered the lumen of the tubules, or the whole necrotic mass of host cells together with parasites was detached from the basic membrane and pushed into the lumen (Fig. 9). Pseudo-plasmodia that had emerged from host cells, or that had been released together with epithelial cells, continued development in the lumen of the renal tubules and the ureters, where 2 to 8 spores may develop in a plasmodium (Fig. 10). I suspect that further division of the plasmodia can occur during this coelozoic stage. The development of *Hoferellus cyprini* and *Myxobilatus legeri* are characterized by an annual cycle, with spores formed only during the winter and in the early spring. In the course of development of *Hoferellus gilsoni* (Debaissieux, 1925), a parasite of the eel, pseudoplasmadia forming 4 to 8 spores were located in the urinary bladder where they attached firmly to the

Figs. 5 to 8. Fig. 5. *Nephrocystidium pickii* surrounded by a connective tissue capsule in the kidney of pike. This large xenoma formation contains degenerated trophozoites of *Myxidium lieberkuehni* (t), enlarged nucleus of the host cell (n) and fragments of the glomerulus (g). Excreted plasmodia (arrow) occur in the renal tubules. H&E staining. Scale bar = 110 µm. Fig. 6. *Myxidium rhodei* plasmodium (p) containing sporogonic stages and spores in a Bowman’s capsule in roach kidney; the rest of the glomerulus (g) has been compressed and pushed aside. H&E staining. Scale bar = 40 µm. Fig. 7. Cross-section of a renal tubule from rudd kidney. Trophozoites of *Myxobilatus legeri* (arrows) are located inside epithelial cells. H&E staining. Scale bar = 10 µm. Fig. 8. Developing *Hoferellus cyprini* (Doflein, 1898) trophozoites (t) in the epithelial cells of the enlarged tubular wall. Uninfected epithelial cells (arrow) are on the other side of the channel. Pseudoplasmidia of *Sphaerospora renicola* (s) are located inside the tubular lumen. H&E staining. Scale bar = 40 µm
urothelium (Fig. 11). Based on the development of other species of *Hoferellus*, the existence of an early intercellular stage in the urinary channels cannot be excluded for this species either.

**Intercellular development among haematopoietic cells**

Dispersed pseudoplasmodia in the renal interstitium among haematopoietic cells are characteristic for some species of *Sphaerospora* and *Tetracapsuloides bryosalmonae* (Canning et al., 1999), the causative agent of proliferative kidney disease of salmonids. In both cases pseudoplasmodia were dispersed among the haematopoietic cells (Fig. 12).

In *Sphaerospora tincae* Plehn, 1925, stages found in the head-kidney represented the final stage of sporogonic development, and they reached this location most likely after a presporogonic phase in the blood. *Tetracapsuloides* trophozoites multiplied among haematopoietic cells by several internal cleavages. In *T. bryosalmonae*, the intercellular stage was only a part of the development, which continued in the renal tubules until spore formation. It is still not known exactly how intercellular stages are transferred to the renal tubules. A special form of intercellular development characterized *Sphaerospora colomani* Baska, 1990 for the intercellular stages were composed of a structure containing 30 to 40 globules. Although these structures (and the trophozoites of *Tetracapsuloides*) seemed to be located among haematopoietic cells,
there remains the possibility that they were actually located in small capillaries that densely interlace this tissue.

**Formation of myxozoan stages in large plasmodia**

Large groups of myxospores were commonly seen in the interstitial tissue of the kidneys outside tubules and renal corpuscles. Two types of these spore conglomerations can be distinguished. In the first, large plasmodia developed among haematopoietic cells (Figs. 13 & 14), whereas in the second case aggregations of spores occurred within the melano-macrophage centres (Figs. 15 & 16), apparently arriving from other organs, such as muscle. The 2 ‘cyst-like’ formations could easily be distinguished during the early stage of plasmodial development. *Myxobolus* plasmodia in this stage contained multinucleated pansporoblasts as well as immature spores (Fig. 13). In this stage of development, the blood vessel in which the plasmodium developed was only partially blocked and erythrocytes were situated between the endothelium of the vessel and the ectoplasm of the plasmodium. More mature plasmodia were filled with hundreds of spores, and bordered by a single cell layer of connective tissue (Fig. 14). However, a connective tissue capsule composed of ≥2 layers was often formed around the plasmodia. In this stage of the development, it was difficult to differentiate between true plasmodia and simple
aggregations of spores within melano-macrophage centres (Fig. 15). These simple aggregations could be recognised by the presence of macrophage cells or the melanin pigments they contained (Fig. 16).

Alterations in the kidney caused by myxozoan stages

Both coelozoic and histozoic stages caused noticeable kidney pathology and frequently caused disease. Large plasmodia such as *Nephrocystidium pickii* or plasmodia of various *Myxobolus* spp. mechanically damaged surrounding tissues by compaction. Interstitial stages, e.g. trophozoites of *Tetracapsuloides bryosalmonae* enlarged the volume of the kidney causing proliferative kidney disease in salmonids. Degenerated spores within melano-macrophage centres may cause malfunction in the kidneys. In hoferelliosis, which is caused by *Hoferellus carassii* Akhmerov, 1960 in goldfish and by *H. cyprini* in common carp, the infected epithelial cells became detached from the *membrana basialis* in the affected part of the renal tubules (Fig. 9), and necrotic cells with plasmodia accumulated in the lumen of the convoluted tubules. Due to severe epithelial damage, kidney enlargement disease is fatal for diseased goldfish. In *Sphaerospora tinciae* infection of tench, the enlargement and swelling of the head kidney were the major clinical signs. The glomerular stages of *Myxidium lieberkuehni* in pike, *M. rhodei* in cyprinids, and *M. giardi* Cépede, 1906 in eel may destroy most of the renal corpuscles and evoke oedema. Relatively little pathology was attributable to sporogonic development of *Sphaerospora* stages in the convoluted tubules, but presporogonic stages of these parasites may cause damage in other organs, e.g. swim-bladder inflammation by *S. renicola* infection.

**DISCUSSION**

Myxozoans developing in fish kidneys show a high affinity for different functional regions of this organ. Some are coelozoic in the lumen of the urinary ducts, others develop in the tubular epithelium, the endothelium of the renal corpuscles, or the haematopoietic tissue of the renal interstitium. For other myxozoans, the kidneys serve only as a depot, where spores from the bloodstream are collected, stored and destroyed by melano-macrophage centres in the interstitium. All types of tissues in the kidneys can be infected by myxozoan parasites. Five major forms of renal infections can be distinguished, viz. (1) coelozoic stages in the renal tubules, (2) plasmodial stages in the renal corpuscles, (3) intraepithelial stages in the wall of the urinary channels, (4) disseminated small pseudoplasmodia in the haematopoietic tissue, and (5) large plasmodia in the haemopoietic tissue. A combination of these major types can also occur.

Of myxozoans developing in the renal tubules, *Sphaerospora* spp. are the most common. Since Csaba et al. (1984) described the development of *S. renicola*, it has been known that *Sphaerospora* spp. have one or 2 blood stages during their presporogonic development, and that the renal phase is only a short phase during development. But whereas the blood stages are regarded as highly pathogenic parasites, the renal stages attaching to the wall of the urothelium or partially obstructing the tubules cause only minor changes (Molnár 1980). *Sphaerospora* spp. seem to be rather common in fish kidneys, as they have been described from cyprinids (Lom et al. 1985b), percids (Pronin & Pronina 1985), silurids (Hedrick et al. 1990), salmonids (McGeorge et al. 1996) and also loricaida (Paperna & Di Cave 2001).

For several parasites, only the late sporogonic stages are known. In most cases the coelozoic stage is preceded by an extrasporogonic development in the renal glomeruli or in the epithelium of the urinary ducts. The majority of *Myxidium* spp. have this developmental pathway, with relatively large plasmodia developing in the renal corpuscles. According to Lom et al. (1989), *Myxidium lieberkuehni* begins its development in the renal corpuscles where an early trophozoite of the parasite transforms the infected endothelial cells, and developmental stages fill the cytoplasm of the enormously expanded host cell. Only a huge hypertrophic nucleus indicates the origin of the xenoma-like formation known previously as a separate species, *Nephrocystidium pickii*. Several other species of *Myxidium* follow this manner of development and produce spores in the renal tubules after the initial glomerular phase (Lom & Dyková 1992). Among them, *M. rhodei* is an exception, as the spores may develop inside the Bowman’s capsule. A similar development occurs in *Chloromyxum lenorae* Lom, Dyková & Kepr, 1988 in turbot and in *C. inexpectatum* Baska, 1990 in sterlet (Lom et al. 1998, Baska 1993).

A newly emerged disease of migrating salmonids and marine fishes is caused by infection with *Parvicapsula*. Among them, *P. minibicornis* Kent, Whitaker & Dawe, 1997, which is the best studied species (Kent et al. 1997, St-Hilaire et al. 2002), seems to follow the *Myxidium* type development involving initial glomerular stages.

The complicated development of *Hoferellus cyprini* was recognized by Plehn (1924), who observed that during 12 mo of development this parasite has developmental stages in the epithelium of the tubules and late sporogonic stages in the lumen of the urinary channels. Kovács-Gayer et al. (1987) found that in the
course of the intraepithelial stage of *Hoferellus cyprini* secondary, tertiary and quaternary units are formed by internal cleavage inside the primary parasitic cells. After the parasite is released into the lumen, the secondary cells form the plasmodia, tertiary cells act as pansporoblasts, and quaternary cells are the sporoblasts. A similar development was described by Ahmed (1973) for *Mitraspora cyprini*, the causative agent of kidney enlargement disease in goldfish. Molnár & Kovács-Gayer (1986) reduced the genus name *Mitraspora* to synonymy with *Hoferellus*, and Molnár et al. (1989) regarded *Hoferellus* stages infecting goldfish as members of the species *H. carassii* Akhmerov, 1960. *Myxobilatus legeri* (Cepede, 1905), a parasite of several leuciscid cyprinids, follows the *Hoferellus*-like developmental pathway. Its intracellular plasmodia, however, do not form an uninterrupted infection in epithelial cells; they are irregularly disseminated in host cells (Molnár 1988). A specific location characterizes *Hoferellus gilsoni*, a parasite of eel. Trophozoites of this species have been found only in the urinary bladder (Lom et al. 1986), but the existence of early intraepithelial stages also seems likely.

*Tetracapsuloides bryosalmonae* is significant economically and the best example for myxozoan trophozoites locating intercellularly among haematopoietic cells of the renal interstitium. Trophozoites of this parasite (PKX cells) multiplying with internal cleavage are located free among interstitial cells (Clifton-Hadley et al. 1984, Kent et al. 1994, St-Hilaire et al. 2002), but the possibility that PKX cells are located in small capillaries in the interstitium cannot be excluded. This latter possibility is supported by observations that similar PKX cells occur in other organs as well, and could serve also as an explanation for the route of further development, viz. for the entry of sporogenic stages into the renal tubules through the renal glomeruli. A similar interstitial development occurs in *Sphaerospora colomani* in sterlet (Baska 1993), and groups of dividing presporogenic stages of this parasite occur among haematopoietic cells (op. cit.). The great resemblance of these stages to the second blood stages of *Sphaerospora renicola* suggests, however, that they were indeed located in capillaries of this tissue, and reached the glomeruli via the blood stream. Infection of the head kidney of tench by *Sphaerospora tinciae* differs from the above infection types in that the spores develop among haemopoietic cells. Spores from this organ can reach the outside world either with the help of macrophage activity via the bloodstream, or only after the death of the host (Hermanns & Körting 1985).

When large masses of spores of *Myxobolus, Thelohanellus* or *Henneguya* occur in the renal interstitium bordered by a single or several layers of connective tissue, it is important at the outset to determine whether spores developed in plasmodia inside the kidney or whether they were carried there by the blood stream and captured by macrophage cells. In the case of old infections, it is difficult to provide proper diagnosis. Macrophages in melanomacrophage centres of the kidney and other organs can accumulate large masses of spores from the bloodstream (Roberts 1975, Dyková 1984, Molnár & Kovács-Gayer 1985, Holzer & Schachner 2001). When only a moderate number of spores are found inside these centres, the yellow or black coloured macrophages can be distinguished easily from the spores (Fig. 16). When, however, the number of accumulated spores is large, it is rather difficult to distinguish these centres from the old plasmodia into which macrophages have infiltrated.

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