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Experimental transmission of *Sphaerospora renicola* to common carp *Cyprinus carpio* fry

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ABSTRACT: The causative agent of renal sphaerosporosis and swimbladder inflammation was observed in specific-pathogen-free (SPF) carp *Cyprinus carpio* L. of the year following exposure to filtered water from a pond where *Sphaerospora renicola* Dykova et Lom, 1982 is enzootic. SPF carp showed infected kidney tubules after contact with water filtrates containing particles <45 µm in diameter. Using a Polymon filter the operative diameter of the causative agent could be determined as being <20 µm as shown by distinct *S. renicola* infections in SPF carp after exposure to 20 µm filtrate. Previous reports of a direct infection by contact with spore-releasing donor carp or feeding on spore-containing kidney tissue could not be confirmed. It is hypothesized that an actinospore component may be the causative agent of *S. renicola* infections in common carp.

INTRODUCTION

Since Wolf & Markiw (1984) reported results of infection trials to elucidate the life cycle of *Myxobolus* (*Myxosoma*) *cerebralis* and revealed an actinosporean as the infective organism of whirling disease of salmonids, a new model of the myxosporean life cycle including a corresponding actinosporean has become increasingly accepted. The results of experiments on transmission of other myxosporeans performed by several authors (El-Matbouli & Hoffmann 1989, Hedrick et al. 1989, Kent et al. 1991, Ruidisch et al. 1991, El-Matbouli et al. 1992, Großheider & Körting 1992) have supported this new idea of a common life cycle for formerly separated species of the divided classes of the phylum Myxozoa Grassé, 1960.

The aim of the present study was to clarify whether the new model could be applied to the life cycle of *Sphaerospora renicola* Dykova et Lom, 1982, the causative myxosporean of swimbladder inflammation (SBI) of common carp *Cyprinus carpio* L. fry, or whether, as an alternative hypothesis, carp fry become infected directly by uptake of *S. renicola* spores. With reference to the results of Wolf & Markiw (1984) it seems to be impossible to exclude *a priori* any organism living in the pond environment as an intermediate or second host. Therefore we divided the field of work into looking at the pond substrate, investigations on the pond water body, and tests on invertebrates such as molluscs and oligochaetes.

The present paper reports results of experiments on material derived from the water after filtration, and of trials in which acceptor common carp were exposed to kidney tissue containing *S. renicola* spores or to aquarium water contaminated with spores.

MATERIALS AND METHODS

Acceptor common carp *Cyprinus carpio* L. Specific-parasite-free carp fry (SPF carp) from a laboratory-bred stock ranging from 25 to 50 mm in length were used as acceptor carp in all experiments. The experiments were performed in thoroughly cleaned aquaria using tap water. Kidney samples from exposed acceptor carp were examined as fresh preparations and (if necesssary) as smears stained with Giemsa's.

Trials with infected donor carp. All donor carp used in this series of experiments originated from a farm pond in Lower Saxony (NW Germany) which harboured *Sphaerospora renicola* infected carp with a prevalence of about 80 to 100 %.

Two series of experiments were carried out to test *Sphaerospora renicola* infection of carp fry by spore uptake:

During the first set of experiments (Table 1A) donor carp (Trial nos. 5-89 & 14-89) and acceptor carp were kept together in the same aquarium. In further trials, kidney tissues of groups of 8 to 12 highly infected, spore-containing donor carp were pooled and fed in equal portions to between 4 and 11 groups of 2 acceptor carp each in 400 ml glasses (nos. 15-89, 21-89, 22-89 & 08-90). Acceptor carp were then combined 36 h later and kept in 1 aquarium.

In the second series (Table 1B) acceptor carp were maintained in contaminated aquaria where donor carp had previously been kept for 57 (no. 20-89) and 62 (no. 7-90) days. In this series aquarium water was not renewed after removing donor carp.

Trials with planktonic material. In 1990 and 1991, planktonic material was obtained by filtration of pond water from the same pond as before. Volumes of 1300 to 1500 l were taken weekly 50 cm below the surface of the pond water from the 2nd to the 7th week after hatching of the carp fry. The water was passed sequentially through a set of stacked PVC screens with mesh sizes of 630, 300, 212, 125 and 45 µm. Resuspended material collected from these screens was divided into a sample fixed with 4% formalin for diagnostic purposes and into material for infection experiments. In addition, after passing through the 45 µm screen the final filtrate was used in an infection experiment.

Three further infection experiments using filtrates of volume 160 to 180 l which passed first through screens of 45 µm and subsequently through a 20 µm Polymon mesh were carried out in a Sartorius filter holder. The 20 µm filter meshes, covered with trapped material, were also exposed to SPF carp to test the infectivity of the collected material.

RESULTS

Trials with infected donor carp

In experiments carried out to test the infectivity of *Sphaerospora renicola* spores directly taken up by ac-

Table 1. *Sphaerospora renicola* infecting *Cyprinus carpio*. Infection trials without second host. SPF: specific-pathogen-free carp

A. No time interval after releasing spores

Trial no.	Exposure to	SPF total	Day no. of exposure First/last sample	Observation of *S. renicola*, SBI
5-89	Donor carp separated by net	27	13/71	None
14-89	Donor carp not separated	8	30/40	None
15-89	Homogen. kidney of donor carp	10	28/36	None
21-89	Homogen. kidney of donor carp	16	60/89	None
22-89	Homogen. kidney of donor carp	21	69/94	None
8-90	Homogen. kidney of donor carp	7	63/114	None

B. Subsequent exposure after keeping donor carp in aquaria

Trial no.	Tank contamination	SPF total	Day no. of exposure First/last sample	Observation of *S. renicola*, SBI
20-89	29 donor carp for 57 d in 60 l[a] replaced by SPF carp	76	55/232	None
7-90	26 donor carp for 62 d in 60 l[a] replaced by SPF carp	10	63/86	None

[a]Tank volume

ceptor carp, all of the kidney samples of acceptor carp were negative in microscopical examinations (Table 1). None of the total of 54 SPF carp fed on spore-containing donor carp kidney tissue showed symptoms of either SBI or developmental stages of myxosporeans when dissected and examined microscopically (Table 1A). No renal sphaerosporosis developed in the 39 SPF carp which were kept together with donor carp in the same tank or in the 83 SPF carp which were kept subsequently (Table 1B) in contaminated aquaria.

Trials with planktonic material

None of the experiments using material collected on PVC screens with mesh sizes ranging from 45 to 630 µm led to *Sphaerospora renicola* infected carp or to swimbladder inflammation in SPF carp, as shown by macroscopical and microscopical examinations (Table 2). Therefore species determination of planktonic organisms fixed in 4 % formalin was not carried out.

In contrast, all infection experiments using filtrates which passed through the 45 µm screen were successful in the transmission of *Sphaerospora renicola* to SPF carp (Table 3), and established low to middle degree infections.

The filtrate containing particles <45 µm yielded, in addition to sporoblasts, spores of *Sphaerospora renicola* and SBI. In this test 20 out of 30 carp were infected, 6 showed spores and 5 showed typical signs of SBI in macroscopical examinations. In addition developmental stages of *S. renicola* ('K-cells') could be observed in Giemsa-stained smears from infected swimbladders (Table 3).

Table 2. *Sphaerospora renicola* infecting *Cyprinus carpio*. Infection trials with planktonic material, collected from pond water volumes of 1300 to 1500 l in 1990 and 1991(*); starting 2nd week after hatching of carp. SPF: specific-pathogen-free carp

Week[a]	Mesh (µm)	SPF total	SPF/ 50 d[b]	Day no. of exposure First/last sample	Observations of SBI, sphaerosporosis
	45	30	16	29/126	
	45	13	8	28/56*	
2	125	32	16	28/113	None
	212	12	8	29/56*	
	300	11	8	28/54*	
	45	50	22	24/140	
	125	49	22	23/72	
3	125	12	8	28/57*	None
	212	13	8	28/57*	
	300	12	8	28/56*	
	45	35	29	22/76	
	45	9	8	28/55*	
	125	26	23	21/71	
4	125	12	7	28/55*	None
	212	10	7	28/55*	
	300	39	27	21/76	
	300	11	8	28/55*	
	630	19	15	21/65	
	45	23	20	30/133	
5	125	35	21	29/132	None
	300	30	25	30/133	
	630	23	20	30/140	
	45	16	14	31/148	
	125	21	15	30/97	
6	212	21	15	30/131	None
	300	24	15	30/135	
	630	25	16	30/147	
	45	15	11	26/79	
7	125	18	15	37/83	None
	212	21	14	38/83	
	300	16	13	38/84	

[a] Week after hatching of carp; [b] no. of carp dissected in the first 50 d of exposure

Table 3. *Sphaerospora renicola* infecting *Cyprinus carpio*. Infection trials with filtrates of pond water containing particles < 45 µm in diameter, volumes 160 to 180 l. SPF: specific-pathogen-free carp

Week[a]	Mesh (µm)	SPF total	Day no. of exp. First/last sample	Kidneys infected Total	Spores	SBI m[b] / K[c]
8	< 45	30	22/126	20	8	5 / 5
13	< 45, > 20	40	21/61	9	0	0 / 0
13	< 20	42	21/77	17	7	6 / 4
13	< 20	37	21/54	13	4	3 / 3

[a]Week after hatching of carp
[b]Macroscopically
[c]K-cells in Giemsa-stained smears

Kidneys obtained from SPF carp exposed to 20 µm Polymon meshes showed cells and cell groups with typical myxosporean features with a prevalence of 9 carp out of 40. However these were rarely detectable in Giemsa-stained smears and in this experiment it was not possible to demonstrate *Sphaerospora renicola* spores or inflamed swimbladders in the acceptor carp.

In the 2 tests using water containing particles with an operative diameter < 20 µm, SPF carp developed *Sphaerospora renicola* infections at prevalences of 17 carp out of 42 and 13 carp out of 37 respectively; *S. renicola* spores and sporoblasts were observed. In addition, in both experiments some SPF carp showed macroscopically an inflammation of the swimbladder and 'K-cells' in Giemsa-stained preparations (Table 3).

DISCUSSION

Trials with infected donor carp

The first series of infection trials sought to confirm both the direct life cycle of myxosporeans commonly understood before Wolf & Markiw (1984) reported the first actinosporean/myxosporean model, and the results of Odening et al. (1989), who reported several successful transmissions of *Sphaerospora renicola* without a second host.

In tests carried out by Odening et al. (1989), acceptor carp were kept either together with donor carp harbouring *Sphaerospora renicola* spores, or in aquaria where infected carp had previously been kept. In further tests, acceptor carp were fed on *S. renicola* infected kidney tissue. In Odening et al.'s experiments acceptor carp infected with *S. renicola* spores were obtained. Spores were detected 10 wk or more after starting the tests, with low prevalences of 1 carp out of 14, 3 carp out of 16, and 2 carp out of 22 respectively for the 3 types of test.

In our investigations we tried to confirm Odening et al.'s results, following his time schedule of sampling kidney tissue of acceptor carp, but our efforts to induce *Sphaerospora renicola* infections failed (Table 1A, B).

Trials with planktonic material

The water used for infection experiments was obtained from a well-studied pond, where the course of *Sphaerospora renicola* infections in common carp populations hatched at the farm has been monitored since 1979. Biffar (1990) reported that *S. renicola* infections became apparent in carp originating from this pond from the 5th to 6th week and spores were detectable from the 7th to 8th week after hatching. These results correspond to those of several other authors (Körting & Hermanns 1984, Grupcheva et al. 1985, Odening et al. 1989). In 1990 Meyer (1991) determined the time interval until the sphaerosporosis-causing agent had infected carp fry in the monitored pond as 16 to 18 d after hatching. Thus in 1990 the causative agent of *S. renicola* infections must have been abundant in the farm pond in the 3rd to 7th weeks after hatching of carp.

For these reasons filtration of pond water was started in the 2nd week after hatching when a high level of infectivity could be expected. The negative results of infection trials using material collected on screens with mesh sizes > 45 µm could not be explained by an insufficient water volume (1300 to 1500 l) because the positive results of infection trials using planktonic material with effective diameters < 45 µm prove that pond water volumes of 160 to 180 l contain sufficient infective agents. Therefore the reason that the causative agent of *Sphaerospora renicola* infections was not trapped on the screens with meshes ranging from 45 to 630 µm in size is clearly that the agent is < 45 µm in diameter.

Experiments with filtrates which had passed through membranes with mesh size 20 µm led to a distinct *Sphaerospora renicola* infection and in several cases to SBI and therefore proved that the size of the operative diameter of the causative agent of SBI is < 20 µm. These data correspond to the work of Hedrick et al. (1992) who demonstrated that water apparently containing the causative agent (PKX) of proliferative kidney disease (PKD) was infective to *Oncorhynchus mykiss* after filtration through a screen with a mesh size of 50 µm. Thus an operative diameter < 50 µm for the PKX organism was assumed.

In spite of the size of the spore of *Sphaerospora renicola*, ranging from 6 to 8 µm in diameter, the results of

the experiments on direct infection make it improbable that *S. renicola* infections in a pond are caused by a planktonic *S. renicola* spore. Moreover the density of some myxosporean spores has been determined at >1.0 g ml^{-1}, e.g. 1.062 g ml^{-1} for *Henneguya doori* (McConnell & Cone 1992) or 1.080 g ml^{-1} for *Myxobolus cerebralis* (Hamilton & Canning 1988). This causes the spores to settle to the bottom of the pond and is therefore in contradiction to the idea of a planktonic myxosporean spore.

Recently an increasing number of myxosporean species have had an oligochaete revealed as a second host in their life cycle, thus actinosporeans parasitizing these oligochaetes may be a phase of the myxosporean development (Wolf & Markiw 1984, El-Matbouli & Hoffmann 1989). This even includes species of the genus *Hoferellus* (El-Matbouli & Hoffmann 1992, Großheider & Körting 1992) which belong to the family Sphaerosporidae Davis, 1917 (Lom 1986).

Bearing in mind these observations, an actinosporean spore released from an unknown oligochaete could be expected to be the causative agent of *Sphaerospora renicola* infections. However, known actinosporean spores are >20 µm in diameter so it is difficult to imagine that a complete actinosporean spore is the planktonic agent of the *S. renicola* infection.

Following the myxosporean/actinosporean model it seems useful to look for a smaller component of the actinospore such as the sporoplasms located in the epispore and found in the skin of rainbow trout *Oncorhynchus mykiss* at the beginning of invasion by *Myxobolus cerebralis* (Markiw 1989). By comparison, Marques & Ormieres (1982) observed disintegration of the spores of *Synactinomyxon longicauda* (Myxozoa: Actinosporea) and the spontaneous releasing of numerous sporoplasms after leaving its oligochaete host and contacting the surrounding water. Thus the sporoplasm of an unknown actinosporean should be considered as a hypothetical planktonic agent of *Sphaerospora renicola* infections of common carp fry.

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