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Cross-infection Experiments Confirm the Host Specificity of *Goussia* spp. (Eimeriidae: Apicomplexa) Parasitizing Cyprinid Fish

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Summary. The host specificity of the coccidian *Goussia carpelli* (Léger *et* Stankovitch, 1921) (Eimeriidae: Apicomplexa) was studied in aquarium experiments. Oocysts were obtained from the gut of 1- to 2-year-old common carp intensively infected with *Goussia carpelli*. These oocysts were mixed into mud containing infection-free oligochaetes (*Tubifex tubifex* and *Limnodrilus hoffmeisteri*). Laboratory-cultured fish demonstrated to be infection free were infected by feeding oligochaetes. The susceptibility of 3-5 cm long fingerlings of 8 fish species [common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), barbel (*Barbus barbus*), bleak (*Alburnus alburnus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), white bream (*Blicca bjoerkna*) and vimba (*Vimba vimba)*] was experimentally evaluated. In the gut of common carp, intensive infection developed each of eight experiments, and oocysts were consistently detectable in the faeces and gut scrapings on days 11-20 after feeding the fish with the oligochaetes. At the same time, oocyst formation could not be demonstrated in the gut of the other seven cyprinids. In another experiment of similar design, only the goldfish could be infected with oocysts obtained from naturally infected goldfish, and no infection was established in the common carp. The results of these experiments suggest that *G. carpelli* is strictly specific to common carp, while *Carassius* species most closely related to the common carp are parasitised by a distinct species of *Goussia* with which it shares morphological similarity.

Key words: Apicomplexa, coccidia, cyprinid fishes, experimental infection, *Goussia carpelli*, *Goussia* sp., oligochaetes.

INTRODUCTION

Goussia carpelli, described by Léger and Stankovitch (1921) from common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*), is one of the longest known and best studied fish coccidians. This parasite is a typical example of gut-parasitic fish coccidia of small size, not exceeding 14 µm in diameter, and having a compact oocyst. Of the closely related known species described from cyprinids, *G. legeri* Stankovitch, 1920, *G. cyprinorum* (Stankovitch, 1921), *G. cylindrospora* (Stankovitch, 1920), *G. cheni* (Chen, 1956), *G. sinensis* (Chen, 1956) and *G. iroquoina* (Molnár *et* Fernando, 1974) have similar intrapiscine location and structure. Although the majority of these coccidian species had been described under the name *Eimeria*, Dyková and Lom (1981) proposed that they should be classified in the genus *Goussia* created by Labbé (1896) because their sporocysts consist of two valves connected by central sutures.

Stankovitch (1920, 1921) had previously suggested that oocysts isolated from different cyprinids repre-

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sented distinct parasite species, a view subsequently advocated by Belova and Krylov (2000) who suggested that the host-specificity of fish coccidia is as strong as that of coccidia parasitising terrestrial vertebrates. Other authors (Musselius and Laptev 1967, Shulman 1984), however, were of the opinion that certain species, such as *G. carpelli*, were able to develop in numerous cyprinid hosts. Moreover, Shulman and Zaika (1964) claimed to have found *G. carpelli* even in a fish species belonging to the Cottidae family. Specificity experiments using *G. iroquoina*, a *G. carpelli* type coccidium species, have been performed by Paterson and Desser (1982). The most reliable experimental results were obtained by Lukeš *et al*. (1991) who described that, with the exception of the goldfish, no infection could be produced in 9 other cyprinid fishes with *G. carpelli* originating from the common carp.

The objective of this experimental work was to determine which cyprinid fish species, other than the common carp, can be infected successfully with *G. carpelli* oocysts originating from common carp.

MATERIALS AND METHODS

Fish and oligochaetes used for experiments. Three- to 5-cm-long fingerlings of cyprinids, hatched in laboratory and reared in parasite-free environment, were used in the experiments. In addition to common carp (*Cyprinus carpio*), fingerlings of goldfish (*Carassius auratus*), barbel (*Barbus barbus*), bleak (*Alburnus alburnus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), white bream (*Blicca bjoerkna*) and vimba (*Vimba vimba*) were studied. For the transmission of infection, laboratory-cultured, parasite-free *Tubifex tubifex* and *Limnodrilus hoffmeisteri* oligochaetes were used.

Coccidians used for experiments. Oocysts of *G. carpelli* were obtained from the gut of common carp fingerlings and two-summer common carp originating from fish farms. These were dissected a day or two after their transfer to the laboratory, after their gut contents had been excreted. Oocysts present in the mucus covering the intestinal mucosa were maintained in tap-water until the infection of oligochaetes. In each experiment, 10,000-20,000 oocysts were poured into plastic dishes containing 100 oligochaetes and sterilised mud. Similar procedures were used in one of the experiments employing *G. carpelli*-like oocysts obtained from goldfish.

Experimental design for infecting fish. A total of 9 experiments were performed. In the first 8 experiments, oligochaetes infected by *G. carpelli* oocysts were fed to fish. Experiments 1 and 2 were designed to determine the interval, after ingestion, when maximum oocyst excretion occurred. Experiments 4 and 5 were designed to learn how long the infected oligochaetes could transmit *G. carpelli* infection to fish. The objective of experiments 3-7 was to learn whether cyprinids other than the common carp could be infected with *G. carpelli* (Table 1). In experiment 8 the effect of repeated oligochaete feeding on the intensity of infection was studied (Table 2). In experiment 9, oocysts intended for infection were collected from the gut of goldfish fingerlings, and these oocysts were mixed into the mud of the dish containing the oligochaetes (Table 3). Until the conclusion of the experiments, the oocysts collected from common carp and goldfish were regarded as the spore stages of a single species, *G. carpelli*.

Infection of fish by oligochaetes. Oligochaetes were fed to the fish at different post-exposure intervals. The times of infection of oligochaetes and fish are indicated in Tables 1-3. Oligochaetes cut into small pieces were used for infecting the fish. Two to three days before the infected oligochaetes were fed to them, individual fasted fish were in a plastic dish containing 0.5 l of water. These fish, sustained on granulated dry fish food, had previously been acclimated to the consumption of coccidia-free oligochaetes. Infected oligochaetes were fed for one to three days; the duration of feeding varied by experiment. The fish were kept in an individual dish throughout the experiment to limit potential sources of exposure. Throughout the experiment, fish were fed the granulated fish food. Water in the dishes was changed daily and kept fresh and replenished with oxygen by aeration. Relatively few fish were assayed owing to the demands required to maintain fish individually in separate dishes. The experiments were carried out at room temperature (21 to 25°C). The fish were killed 10-21 days after infection, when peak oocyst excretion was expected. Feeding of fish food was stopped two days before the fish were killed.

Examination of fish for infection. The gut of the fish was removed in its entirety, cut open lengthwise, and the mucus in which the oocysts were most easily detectable was lifted off the gut wall as far as possible. In a negative case, besides the mucus the lumps of faeces present in the gut and mucosal scrapings taken from different segments of the intestinal wall were also placed on a slide, under a coverslip. The samples were examined under microscope at 200- to 400-fold magnification. In the case of fish of smaller size, the entire gut was examined for the occurrence of oocysts or developmental stages. For histological examination, segments from the intestine were fixed in Bouin's solution and embedded in paraplast. The 5 µm sections were stained with haematoxylin and eosin solutions. Photomicrographs of fresh oocysts in mucus under a coverslip were taken with an Olympus DH-10 digital camera mounted on an Olympus BH2 microscope.

RESULTS

Experiments 1 and 2, in which parasite-free common carp fingerlings were infected with *G. carpelli* oocysts collected from common carp through tubificid vectors, have confirmed that *G. carpelli* infection can be successfully transmitted to the common carp. Oocysts enclosed in yellow bodies could be detected in the mucus, faeces and gut epithelium at room temperature by days 11-20 in these fish (Table 1; Figs 1, 3).

Equivalent experiments demonstrated that parasites derived from the common carp infections did not result in infections of either goldfish or other cyprinid fishes (Table 1).

Table1. Experimental infection of common carp and other cyprinids with *Goussia carpelli* through oligochaete vectors exposed to oocysts for varying periods.

Fish species	No. fish	Oligochaetes exposed to oocysts before infection (days)	Time from exposure to killing (days)	Number of infected fish	Intensity of infection	No. exp.
Common carp	2	4	14	2	+/+++	4
	1	15-17	12	-	-	5
	1	15-17	17	1	+	5
	2	26	14	2	+/++	4
	1	32	9	1	+++	7
	8	32	12	8	+/+++	7
	1	34	11	1	+	1
	2	34	19	2	+++	1
	2	34	20	2	++	1
	4	31-32	15-16	3	+/+++	6
	4	31-32	15-16	2	++	6
	1	46-48	12	-	-	5
	1	46-48	17	1	+	5
	1	68-70	12	-	-	5
	1	68-70	17	1	+	5
	2	72	14	1	+	4
	2	73-74	12	-	-	2
	2	73-74	13	1	+++	2
	2	73-74	14	1	+++	2
	2	73-74	15	1	+++	2
	4	73-74	16	4	++/+++	2
	2	73-74	19	1	+++	2
	2	73-74	20	2	+/++	2
	2	73-74	21	2	+/++	2
Goldfish	2	31-32	15-16	-	-	6
	2	32	9	-	-	7
	2	4-72*	14	-	-	4
	1	15-70*	12	-	-	5
	1	15-70*	17	-	-	5
Common bream	2	32	12	-	-	7
	2	4-72*	14	-	-	4
	2	31-32	15-16	-	-	6
	1	15-70*	12	-	-	5
	1	15-70*	17	-	-	5
Roach	2	32	12	-	-	7
	2	4-72*	14	-	-	4
	2	31-32	15-16	-	-	6
	1	15-70*	12	-	-	5
	1	15-70*	17	-	-	5
Vimba	2	32	12	-	-	7
	2	31-32	15-16	-	-	6
	2	4-72*	14	-	-	4
	1	15-70*	12	-	-	5
	1	15-70*	17	-	-	5
White bream	2	32	12	-	-	7
	2	31-32	15-16	-	-	6
Bleak	2	32	12	-	-	7
	2	31-32	15-16	-	-	6
Barbel	2	32	12	-	-	7
	2	31-32	15-16	-	-	6
	2	4-72*	14	-	-	4
	1	15-70*	12	-	-	5
	1	15-70*	17	-	-	5

+ - a few oocysts in the gut; ++ - easily detectable coccidian infection; +++ - intensive infection; * - feeding by mixed oligochaete groups exposed to oocysts of varying times

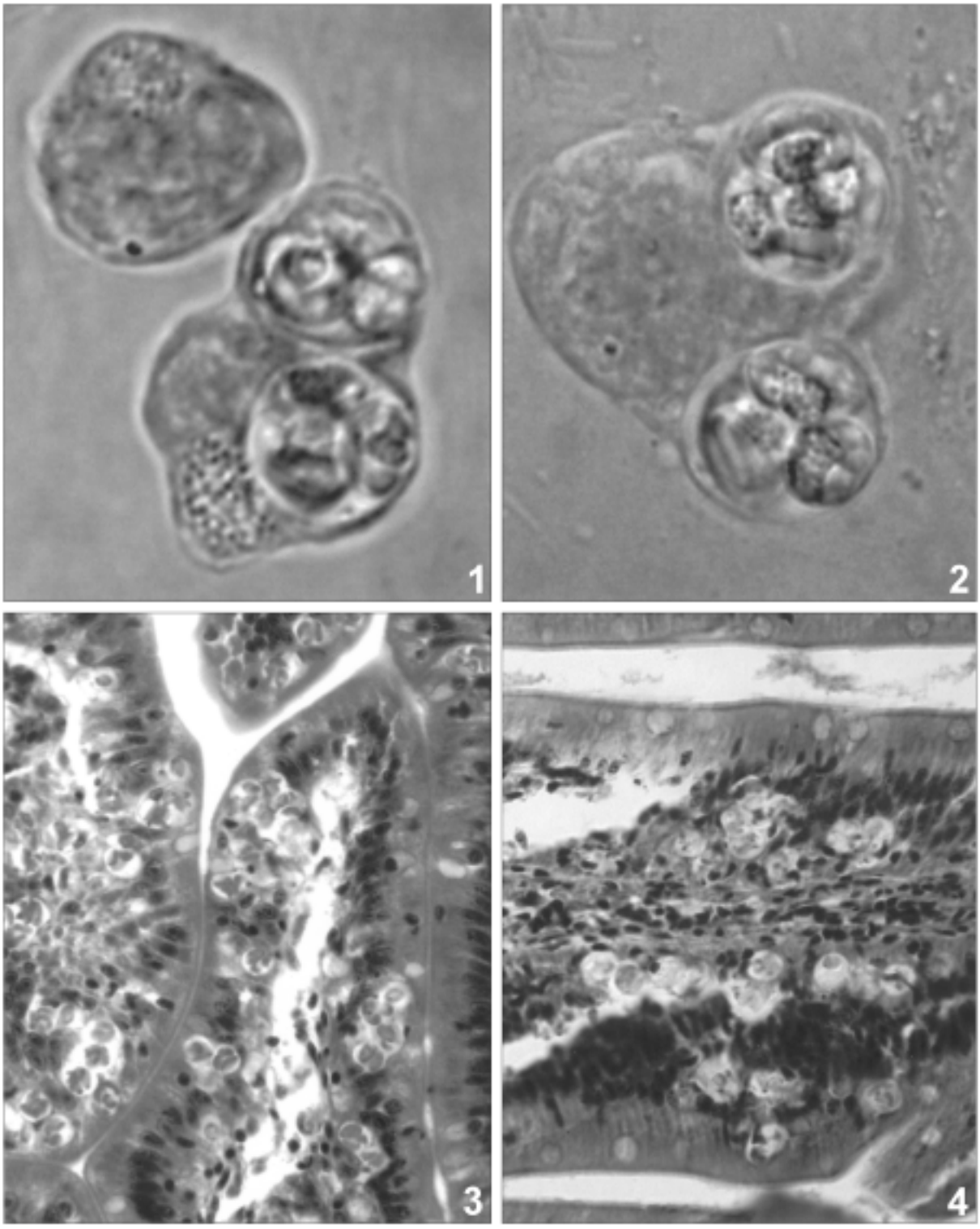

Fig. 1. *Goussia carpelli* oocysts located within a yellow body from the gut of an experimentally infected common carp fingerling. 3000×.
Fig. 2. *Goussia* sp. oocysts located within a yellow body from the gut of an experimentally infected goldfish fingerling. 3000×.
Fig. 3. Sporulated *G. carpelli* oocysts in the gut epithelium of the common carp. Histological section stained with haematoxylin and eosin. 500×.
Fig. 4. Oocysts of a *Goussia* sp. in the gut epithelium of a goldfish. Histological section stained with haematoxylin and eosin. 500×.

Table 2. Common carp and goldfish fingerlings fed for 1-10 days on oligochaetes exposed to *G. carpelli* oocysts.

Fish species	No. fish	Oligochaetes exposed to oocysts before infection (days)	Duration of oligochaete feeding (days)	Time from the first exposure to killing (days)	Number of infected fish Carp/Goldfish	Intensity of infection Carp/Goldfish	No. exp.
Common carp/goldfish	1/1	36	1	15	1/-	++ / -	8
	1/1	36-37	2	15	1/-	++ / -	8
	1/1	36-38	3	15	1/-	++ / -	8
	1/1	36-40	5	15	1/-	++ / -	8
	1/1	36-41	6	15	1/-	++ / -	8
	1/1	36-42	7	15	1/-	+++ / -	8
	1/1	36-43	8	15	1/-	+++ / -	8
	0/1	36-44	9	15	-/-	/ -	8
	0/1	36-45	10	15	-/-	/ -	8

Table 3. Coccidian infection of goldfish and common carp fingerlings after feeding on tubificid oligochaetes exposed to oocysts of a *Goussia* sp. originating from goldfish.

Fish species	No. fish	Oligochaetes exposed to oocysts before infection (days)	Time from exposure to killing (days)	Number of infected fish Goldfish/Carp	Intensity of infection Goldfish/Carp	No. exp.
Goldfish/common carp	3/3	14	9	-/-	-/-	9
	3/3	14	14	3/-	+ to ++/-	9
	2/2	14	20	2/-	+++/-	9
	2/2	14	23	2/-	+++/-	9

The transmission of *G. carpelli* infection from common carp to common carp was successful regardless of whether the oligochaetes had been infected 4, 26, 69 or 72 days before the infection of fish. Oligochaetes infected for longer intervals resulted in less intensive infections in common carp (Table 1).

Oocyst excretion was observed, once, as early as 9 days after infection but regularly from day 13 onwards (Tables 1, 2).

An only minimal increase in the intensity of infection was observed in common carp fed with the oligochaetes for 8 days as compared to those fed oligochaetes for one day (Table 2).

Fish could also be infected through tubificid vectors with oocysts, morphologically appearing identical to *G. carpelli*, collected from goldfish. However, in this case infection developed only in goldfish, while common carp infected in the same way, and serving as controls for the goldfish, remained free of infection (Table 3; Figs 2, 4).

In goldfish, oocyst excretion could also be observed from day 13 post infection (Table 3). At that time the majority of oocysts were still in unsporulated or half-sporulated state in the gut lumen. However, after day 19 an advanced stage of sporulation was observed (Fig. 4).

DISCUSSION

The primary objective of the experiments was to study the host specificity of *G. carpelli* and the possibility to transmit parasites derived from common carp to other cyprinids. To maximize our ability to detect such heterologous infections, we first sought to determine, without robust replication, other transmission characteristics such as the optimum time of infection with oligochaetes and the duration of oocyst formation and oocyst excretion.

As shown by the data of experiments presented in Tables 1-2, *G. carpelli* infection could consistently be

established in infection-free common carp through the feeding of tubificid (*Tubifex, Limnodrilus*) paratenic hosts. Oocyst formation and excretion commenced on days 9-12 post infection and continued at least for further eight days. In all the eight experiments, oocysts could be demonstrated only in common carp in the given period, and other cyprinids remained infection free. It was especially striking that the goldfish did not become infected either, although according to the original description *G. carpelli* is a common parasite of the genus *Cyprinus* and *Carassius* (Léger and Stankovitch 1921, Lukeš *et al*. 1991) could also produce a low-level infection in goldfish during their experiments. In contrast to these results, our experiments suggested that the common carp and the goldfish were infected by two distinct species, and that the common carp could not be infected successfully with oocysts collected from goldfish (Table 3). Otherwise, the intraoligochaete and intrapiscine development of the latter species appeared to be similar to that of *G. carpelli*, and oocyst excretion was recorded from day 13.

The results obtained in this study support the hypothesis that fish coccidia are characterized by relatively strict host specificity (Stankovitch 1921, Léger and Stankovitch 1921, Musselius *et al*. 1965). According to Belova and Krylov (2000), fish coccidia can develop normally only in closely related fish species, but we observe an even greater degree of host specificity in *G. carpelli*, as it cannot cause infection even in those species of *Carassius* most closely related to the common carp, i.e. the goldfish (*Carassius auratus*) and possibly the crucian carp (*C. carassius*) and the gibel carp (*C. auratus gibelio*). The close relationship between these fish species and the common carp is confirmed by morphological characteristics as well as by molecular evidence (Zardoya and Doadrio 1999). Similarly, the common carp cannot be infected with the *Goussia* species collected from goldfish, which, however, can successfully be transmitted experimentally to infection-free goldfish. These facts suggest that the *G. carpelli*-like species parasitising the goldfish might be regarded as a new, hitherto undescribed species. Waiting for the definitive description, this species has been designated as *Goussia* sp.

In these experiments, infection was consistently attempted by the use of oligochaete paratenic hosts. Namely, both in our own experience and according to the findings of Steinhagen and Körting (1990), this is the only method by which *G. carpelli* infection can be reproduced consistently. To date, few authors have attempted to transmit coccidian infections experimentally in fish. Successful infection through direct transmission of oocysts has been reported by Musselius *et al*. (1965), Zmerzlaya (1966), Steinhagen and Körting (1988, 1990) and Steinhagen *et al*. (1989), who conducted their experiments with *G. carpelli* in common carp. Marincek (1973) and Steinhagen (1991) could also successfully infect the same fish species with oocysts of *G. subepithelialis*. As regards to other fish species, Landsberg and Paperna (1985) successfully infected *Tilapia* by direct transmission of *G. cichlidarum* oocysts in tilapia. The paratenic host is necessary for the successful transmission of infection was first suggested by Molnár (1979), who could not induce *G. carpelli* infection in common carp with oocysts mixed in the food but obtained intensive infection by feeding tubificids collected from the natural environment. Similarly, the necessity of mediator organisms has been reported by Solangi and Overstreet (1980) as well as Fournie and Overstreet (1983), who proved that a natural intermediate host, the grass shrimp (*Palaemonetes pugio*), was needed for the development of *Calyptospora funduli*. Kent and Hedrick (1985), who studied *G. carpelli* infection in goldfish (sic?!), successfully induced infection by the use of vectors (tubificids, grass shrimp). However, Steinhagen and Körting (1990) could induce *G. carpelli* infection both directly and indirectly (by the use of vectors), and they regarded tubificids as paratenic hosts only. Nevertheless, the above authors emphasised that infection via vectors was more likely to succeed. Steinhagen (1991) found *G. carpelli* and *G. subepithelialis* sporozoites within parasitophorous vacuoles in the gut epithelial cells of *Tubifex* and established that they maintained their viability for nine weeks. Fournie *et al*. (2000) detected sporozoites of *C. funduli* in the basal epithelial cells of the gut of shrimp, where they became infective in 5 days.

Cross-infection experiments were first reported by Paterson and Desser (1982), who successfully induced *G. iroquoina* infection in the common shiner (*Notropis cornutus*) and fathead minnow (*Pimephales promelas*) with oocysts collected from the common shiner both by direct infection and through *Tubifex* vectors. On the basis of observations made in ponds, Musselius and Laptev (1967) came to the conclusion that if the common carp, silver carp and bighead stocks are reared together the common carp develops only *G. carpelli* infection, whereas the oocysts of *G. carpelli* also can be found in the gut of silver carp and bighead where they occur together with *G. sinensis* and *G. cheni* which

typically infect these fish species. So far, only *G. sinensis* infection has been recorded in silver carp and bighead in Hungary (Molnár 1976), and the oocysts of *G. carpelli* were not detectable from these fish species. The results of the present experiment suggest that it is unlikely that *G. carpelli* infects other fish than the common carp. We suggest that in the experiment of Musselius and Laptev (1967) *G. carpelli* coccidia may have reached the gut of silver carp and bighead with the food owing to conditions applicable only when fish are reared together in aquaria. To rule out this potential source of error, fish in the present experiments were kept in individual dishes, isolated from fish of the same or other species. The only reliable results on the host specificity of *G. carpelli* were obtained by Lukeš *et al*. (1991) who found that this species was strictly host specific. However, in contrast with our results, besides the common carp the above authors could establish a slight infection in the goldfish as well.

Outside the host oocysts from mammals and birds can remain viable and infective for more than one year (Kheysin 1972). However, according to Musselius *et al*. (1965) oocysts remain viable in water only for 20-22 days. According to our own observations (Molnár, unpublished), the period of oocyst viability is actually much shorter, and paratenic hosts play an important role also in the prolonged maintenance of infectivity. In addition to the data reported by Steinhagen (1991), this is supported also by the results of experiments 3 and 4 of this study. In these experiments, besides relatively recently infected oligochaetes, tubificid worms of an oligochaete stock exposed to oocysts four months earlier were also fed to fish. Both stocks established successful infection, though the level of infection with earlier infected oligochaetes was lower than when recently infected tubificid worms were used.

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