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Complete developmental cycle of *Myxobolus pseudodispar* (Gorbunova) (Myxosporea: Myxobolidae)

Cs Székely, K Molnár and O Rácz

Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary

Abstract

Myxobolus pseudodispar (Gorbunova) is a common parasite of the muscle of roach, *Rutilus rutilus* L., whereas its actinosporean development occurs in two oligochaete alternate hosts. This paper reports the complete developmental cycle of this parasite in the oligochaete alternate host *Tubifex tubifex* and the roach. In laboratory experiments, parasite-free *T. tubifex* specimens were infected by myxospores of *M. pseudodispar* collected from roach in Lake Balaton. Parasite-free roach fingerlings were infected with floating triactinospores (TAMs) released from oligochaetes on day 69 after challenge. Young plasmodia and spores in roach were first recorded on day 80 post-exposure (p.e.). Myxospores collected from experimentally infected roach initiated a new development in *T. tubifex* and the resulting TAMs infected roach. No infection of roach resulted from feeding oligochaetes containing mature triactinospores.

Keywords: alternate hosts, experimental infection, life cycle, *Myxobolus pseudodispar*, Myxosporea, *Rutilus rutilus*, *Tubifex tubifex*

Introduction

As Wolf & Markiw (1984) reported, the development of *Myxobolus cerebralis* Hofer was accomplished through a salmonid fish and the oligochaete *T. tubifex* (Müller), and a number of studies have demonstrated that other myxosporean species also develop through oligochaete and, less frequently, polychaete alternate hosts (El-Matbouli & Hoffmann 1989, 1993; Styer, Harrison & Burtle 1991; El-Matbouli, Fischer-Scherl & Hoffmann 1992; Grossheider & Körting 1992; Benajiba & Marques 1993; Yokoyama, Ogawa & Wakabayashi 1993; Uspenskaya 1995; Trouillier, El-Matbouli & Hoffmann 1996; Bartholomew, Whipple, Stevens & Fryer 1997; El-Mansy & Molnár 1997a,b; El-Mansy, Molnár & Székely 1998; Székely, El-Mansy, Molnár & Baska 1998; Székely, Molnár, Eszterbauer & Baska 1999; Molnár, El-Mansy, Székely & Baska 1999a,b; Eszterbauer, Székely, Molnár & Baska 2000). In most of these studies, authors infected oligochaete or polychaete alternate hosts with myxospores collected from fish and after development in these worms they obtained spores of actinosporean types such as *Triactinomyxon*, *Raabeia*, *Aurantiactinomyxon* and *Neoactinomyxum*. Kent, Whitaker & Margolis (1993) and Yokoyama, Ogawa & Wakabayashi (1995) performed reverse experiments, by infecting fish with actinospores obtained from oligochaetes. Kent *et al.* (1993) infected the sockeye salmon, *Oncorhynchus nerka* (Walbaum), with triactinospores released from the lumbriculid *Stylodrilus heringianus* Claparéde, and these stages developed in the brain of salmon into *Myxobolus arcticus* Pugachev & Khokhlov, while Yokoyama *et al.* (1995) infected goldfish, *Carassius auratus* (L.), with raabeia-type actinospores which transformed in the alternate host *Branchiura sowerbyi* Beddard, into a *Myxobolus* species in the cartilage. This species was described as *M. cultus* Yokoyama, Ogawa & Wakabayashi. The complete developmental cycle (myxosporean and actinosporean phase) is known only for some species. El-Matbouli & Hoffmann (1998) successfully repeated Wolf & Markiw's experiment with *M. cerebralis*, while Ruidisch, El-Matbouli & Hoffmann (1991),

Correspondence *Cs Székely, Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, PO Box 18, Hungary*
(e-mail: szekely@vmri.hu)

Bartholomew *et al.* (1997) and Yokoyama (1997) demonstrated the complete developmental cycle of *M. pavlovskii* Akhmerov, *Ceratomyxa shasta* Noble and *Thelohanellus hovorkai* Akhmerov, respectively.

Myxobolus pseudodispar (Gorbunova), is a common myxosporean of the roach, *Rutilus rutilus* L. The development in fish and pathogenic effects of this species were studied in detail by Baska (1987). Székely *et al.* (1999) studied the development of *M. pseudodispar* in oligochaetes. These authors collected myxospores from the muscles of roach and successfully infected *T. tubifex* and *Limnodrilus hoffmeisteri* Claparéde. They reported that after a 2.5-month prepatent period, triactinospores developed in these oligochaetes.

This paper reports experiments in which triactinospores released from *T. tubifex* after challenge with *M. pseudodispar* myxospores led to myxosporean development resulting in *M. pseudodispar* infection in the muscles of roach.

Materials and methods

Myxobolus pseudodispar myxospores were collected from mature intramuscular plasmodia in heavily infected roach from Lake Balaton and from the Kis-Balaton water reservoir, Hungary. Muscles of roach were squashed between two glass plates. Intramuscular plasmodia were separated from non-infected muscle cells and opened using a needle under a stereomicroscope to obtain myxospores. Laboratory-cultured parasite-free *T. tubifex* were challenged in a 500-mL cup by the addition of 50 000 myxospores. Water in the cups was regularly checked for floating triactinomyxon spores (TAMs). The method used was that described by Székely *et al.* (1999).

Fertilized eggs of roach were collected from Lake Balaton, hatched in aquaria and fed exclusively on *Artemia nauplii* and granulated food to obtain parasite free roach fingerlings.

Four experiments were conducted.

In experiments 1 and 2, 20 and 15 SPF roach fingerlings (3–5 cm in body length), were placed into containers containing approximately 3000 freshly released TAMs for 24 and 20 h, respectively. Fish were killed 80–254 days post-exposure (p.e.) and the complete musculature was checked for the presence of *M. pseudodispar* plasmodia in smear preparations under a light microscope. Myxospores were counted by flattening plasmodia and estimating the spore numbers.

In experiment 3, laboratory cultured parasite-free oligochaetes were infected with *M. pseudodispar* myxospores. When floating TAMs appeared in the water of the container the oligochaetes were washed from the sediment and individually placed into 2-mL cell-well plates (see Székely *et al.* 1999). Heavily infected specimens were selected under a stereomicroscope on the basis of released TAMs. Infected oligochaetes were fed to SPF roach fingerlings. Each fingerling received one heavily TAM-infected oligochaete. The fish were killed 90–215 days p.e. and the complete musculature and kidneys were checked for the presence of *M. pseudodispar* plasmodia in smear preparations.

In experiment 4, the cycle was repeated with experimentally obtained myxospores. *Myxobolus pseudodispar* myxospores were collected from the muscle of fish from experiment 1. One hundred *T. tubifex* were challenged with about 10 000 myxospores of *M. pseudodispar*. When TAMs were released into the water of the containers, 10 roach fingerlings were added for 24 h. Fish in this experiment were killed 77 days p.e. and examined for the presence of plasmodia and myxospores as described previously.

Roach cultured in a closed system under parasite free conditions served as controls. The musculature of five fish from this stock were examined by smear preparations in each experiment as a control.

Histology

When muscle cells on one side of the body of roach from experiments 1 and 2 showed relatively heavy infection with intramuscular plasmodia, the other side of the body was fixed in Bouin's solution for 3 h. The fish were embedded in paraffin wax and 4–6 μm sections prepared and stained with haematoxylin and eosin (H & E).

Results

In experiment 1 (Table 1) *T. tubifex* specimens infected with myxospores of *M. pseudodispar* first released TAMs on day 77 p.e. Release of TAMs increased up to day 95 and then decreased continuously to day 120 p.e. The fish were challenged with TAMs on day 90 p.e. *Myxobolus pseudodispar* developed into plasmodia in the muscle in 55% of the exposed fish. Young plasmodia in the roach muscle harbouring developmental stages were first found 80 days p.e. (Fig. 1). Less frequently, these

Table 1 Challenge of roach fingerlings with floating TAMs of *M. pseudodispar* with an exposure time of 24 h (experiment 1)

Fish no.	Fish length (cm)	No. and development of plasmodia (spores)	Necropsy (days p.e.)
1	3.4		
2	3.6	Three young plasmodia	
3	3.8	–	
4	3.5	One young plasmodium with some spores	
5	3.4	–	
6	3.5	–	
7	3.9	One plasmodium (20)	
8	4.1	Three plasmodia (100)	
9	4.2	Seven plasmodia (1500)	
10	4.0	10 plasmodia (3000)	
11	4.1	–	
12	4.5	Five plasmodia (400)	
13	5.0	20 plasmodia (4000)	
14	5.1	–	
15	4.5	Nine plasmodia (3000)	
16	5.0	–	
17	4.5	–	
18	7.0	–	
19	5.2	11 plasmodia (3000)	
20	5.5	Five plasmodia (500)	
Prevalence		11/20 (55%)	

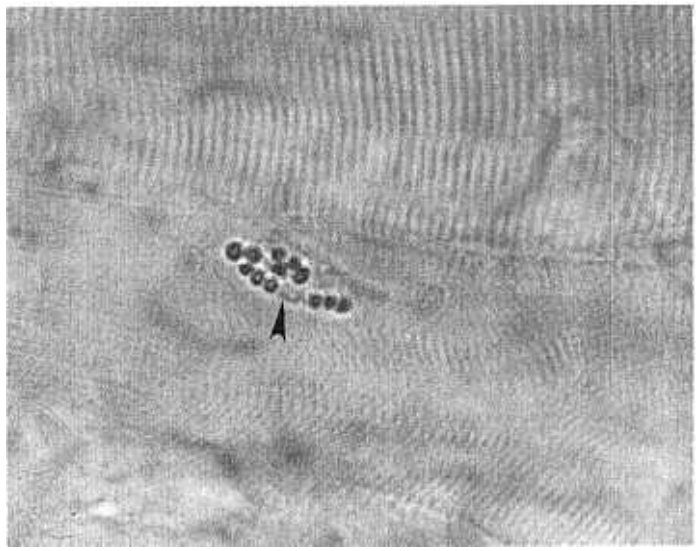

Figure 1 Experimental infection of a roach by *Myxobolus pseudodispar* triactinospores (TAMs) 80 days post-exposure (p.e.). Developing plasmodium (arrow) in one of the muscle fibres (× 1000).

plasmodia also contained some young spores. More developed plasmodia containing mostly spores were found on day 170 p.e. (Fig. 2). Most of the plasmodia were very small (50–120 × 20–40 μm), containing not more than 20–400 myxospores. The number of plasmodia and spores increased with time and some plasmodia reached a size of 300 × 50 μm. In most infected fish only 1–11 plasmodia were found in the muscle; in one roach, however, 20 developing plasmodia were counted. Infection in the challenged fingerlings was found up to day 239 p.e. (8 months). At that time the plasmodia easily released spores on compression of the muscle between glass plates (Fig. 3) and the spores showed the characteristic asymmetric shape of *M. pseudodispar* (Fig. 4). Although plasmodia ruptured easily on compression and spores were released into the intermuscular space, no disseminated spores were found in the kidneys of the roach.

In experiment 2 (Table 2) (which was performed in a similar way as experiment 1) fish were examined only on day 254 p.e., at which time only mature plasmodia with spores were detected. The prevalence of infection was 62%.

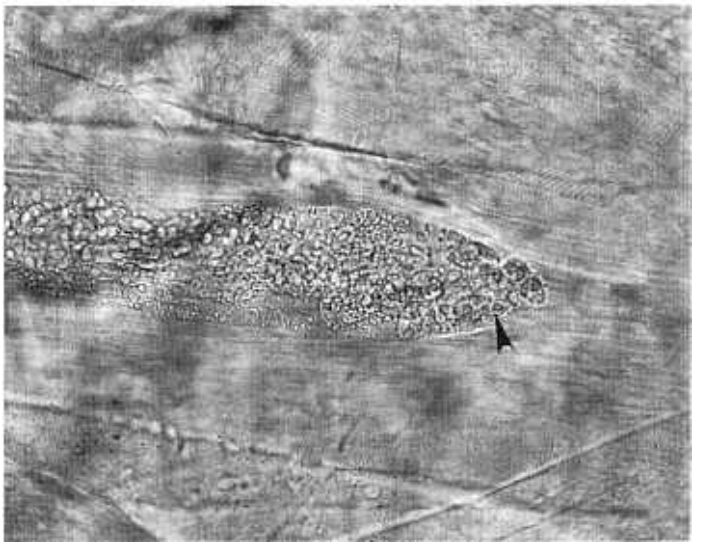

Figure 2 Experimental infection of a roach by *Myxobolus pseudodispar* TAMs 170 days post-exposure (p.e.). A plasmodium containing developmental stages and spores in the muscle fibre (arrow) (× 500).

Figure 3 Myxospores of *Myxobolus pseudodispar* from a squashed muscle plasmodium of an experimentally infected roach 8 months post-exposure (p.e.) (× 500).

In experiment 3, none of the 10 roach fingerlings was found to be infected on days 90–215 p.e. by light-microscopic examination of fresh squash preparations made from the entire musculature and kidneys of the fish. In experiment 4, on day 77 p.e. plasmodia were seen in the muscle cells of 7 of the 10 fingerlings challenged (Table 3). All control fish were uninfected.

Histological examination of the experimentally infected fish showed a typical *M. pseudodispar* infection with intracellular plasmodia in the muscle fibres. Plasmodia fixed in the early stage of infection contained vegetative developmental stages and pansporoblasts (Fig. 5).

Discussion

In the present study, roach and the oligochaete *T. tubifex* proved to be suitable alternate hosts for *M. pseudodispar*. Roach fingerlings reared in the laboratory under parasite-free conditions were successfully infected with experimentally produced floating TAMs of *M. pseudodispar*. After entering the fish host, the infective cells of the sporoplasm of the TAMs developed further and after 2.5 months at 20 °C myxospores were formed. In this experiment, two consecutive complete developmental cycles of *M. pseudodispar* were successfully reproduced by the use of oligochaete and fish alternate

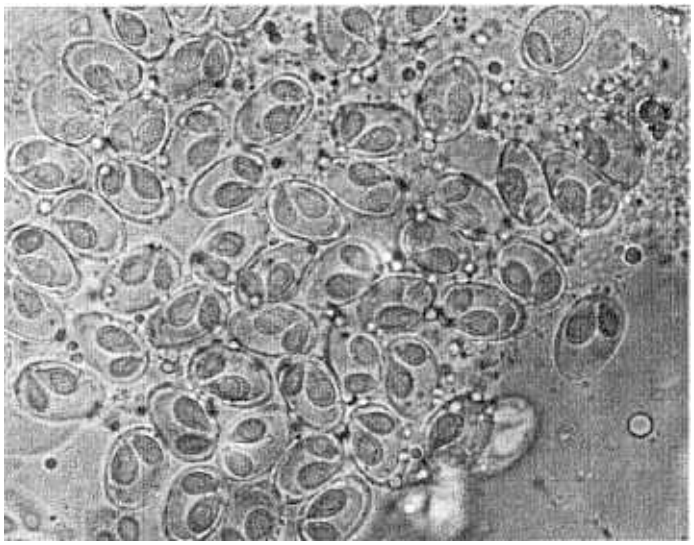

Figure 4 Characteristic asymmetric myxospores of *Myxobolus pseudodispar* released from a mature plasmodium in the muscle of an experimentally infected roach 8 months post-exposure (p.e.) (× 1000).

Table 2 Challenge of roach fingerlings with floating TAMs of *M. pseudodispar* with an exposure time of 20 h and necropsy at 245 days post-exposure (p.e.) (experiment 2)

No. fish	Length (cm)	Infection in muscle	Intensity	Development of plasmodia
1	4.7	+	b	Large plasmodia
2	5.0	–	–	–
3	4.7	+	a	Medium sized plasmodia
4	5.0	+	b	Medium sized and large plasmodia
5	4.4	+	a	Medium sized plasmodia
6	5.7	+	b	Large plasmodia
7	4.6	+	a	Medium sized plasmodia
8	4.5	+	c	Medium sized plasmodia
9	4.7	–	–	–
10	4.7	–	–	–
11	4.9	–	–	–
12	4.7	–	–	–
13	4.0	+	a	One small plasmodium
Prevalence		8/13 (62%)		

Infection in muscle: +: infected, –: non-infected.
Intensity of infection: [a] 1–9 plasmodia, [b] 10–20 plasmodia, [c] > 20 plasmodia.

Table 3 Challenge of roach fingerlings with floating TAMs of *M. pseudodispar* with an exposure time of 20 h and necropsy 77 days post-exposure (p.e.) (experiment 4). TAMs derived from *M. pseudodispar* myxospores obtained from experiment 1

No. fish	Length (cm)	Infection in muscle	Intensity	Development of plasmodia
1	3.0	+	b	Small plasmodia
2	5.8	–	–	–
3	3.8	–	–	–
4	3.4	+	a	Small plasmodia
5	3.0	+	a	Small plasmodia
6	5.6	+	a	Small plasmodia
7	4.5	+	a	Small plasmodia
8	5.2	–	–	–
9	4.2	+	b	Medium sized plasmodia
10	5.0	+	b	Medium sized plasmodia
Prevalence		7/10 (70%)		

Infection in muscle: +: infected, –: non-infected.
Intensity of infection: [a] 1–9 plasmodia, [b] 10–20 plasmodia.

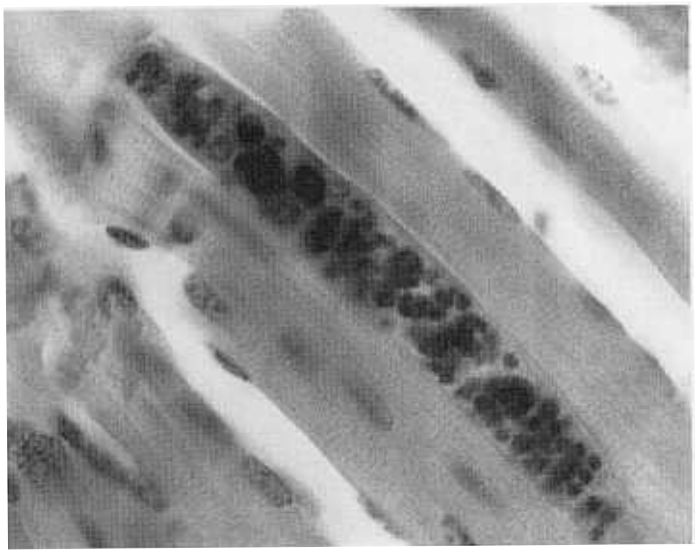

Figure 5 A relatively large *Myxobolus pseudodispar* plasmodium in the muscle fibre of an experimentally infected roach 170 days post-exposure (p.e.) (H & E, × 500).

hosts, which suggests that *M. pseudodispar* infection will be a suitable laboratory model in the future.

In our experiments, infection of the fish occurred only as a result of invasion by floating actinospores and attempts to produce infection by feeding *T. tubifex* specimens infected with mature triactinospores failed. This suggests that in the roach-oligochaete *M. pseudodispar* model the infective cells of actinospores presumably invade the fish through the skin and gills, rather than through the gut wall, and reach the site of final development, the skeletal muscle cells, by a subsequent migration. These results are consistent with the accounts of several authors (Wolf & Markiw 1984; El-Matbouli & Hoffmann 1989, 1998; El-Matbouli, McDowell, Mukkatira & Hedrick 1998), but are at variance with the observations of Yokoyama (1997), who successfully produced *T. hovorkai* infection in common carp, *Cyprinus carpio* L., by feeding *B. sowerbyi* specimens containing aurantiactinomyxon spores.

Although this study has brought us closer to attaining the objective of developing a model suitable for studying the development, ecology and pathological role of myxosporeans, many problems remain unsolved. It is unclear why it was not possible to achieve 100% prevalence of infection despite the use of very high numbers of myxospores and actinospores, and why substantial differences developed in the intensity of infection. Studying the susceptibility of different oligochaete strains, El-Matbouli *et al.* (1998) concluded that within the species *T. tubifex* there are strains of varying susceptibility to infection with *M. cerebralis*; as a result, in some biotopes high prevalence *M. cerebralis* infections develop, while in others the prevalence is low. The differences observed in our experiments cannot apparently be explained by disparities in susceptibility, as both the oligochaete and the fish stocks used were of identical origin and presumably had very similar genetic properties, and the environmental factors, the temperature and the age and number of the spores were also identical.

While the successful infection of oligochaetes and fish undoubtedly requires the presence of a certain number of spores, in this study almost identical results were obtained by the use of a large number of spores collected from naturally infected fish and by the use of a relatively low number of spores derived from experimentally infected fish. In the latter, relatively small plasmodia containing as few as 20–30 spores, a few hundred spores or at most 1000 spores developed, which, however, very successfully infected the fish used in experiment 4. A possible explanation is that the infectivity of young spores not yet damaged by the host reaction substantially exceeded that of spores collected from natural infections, which were probably aged and mostly released from a connective tissue capsule.

No solitary spores were found in the melanomacrophage centres of the kidney in these experiments, although the occurrence of such spores proved to be common in naturally infected fish. The appearance of solitary or aggregated spores surrounded and damaged by macrophages in the melanomacrophage centres of the kidney was found

to be common in severe *Myxobolus cyprini* Doflein, infection by Molnár & Kovács-Gayer (1985) and in *M. pseudodispar* infection of the roach by Baska (1987). According to these authors, this was because spores released into the intermuscular space after the disruption of damaged muscle cells and mature plasmodia were carried to the kidney via the blood stream. The absence of disseminated spores in the experimental material in this study can presumably be attributed to the relatively young age of the spores and to the lower number of spores contained by the small-sized plasmodia.

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