Acta Veterinaria Academiae Scientiarum Hungaricae, Tomus 27 (3), pp. 207—216 (1979)

MYXOBOLUS PAVLOVSKII (ACHMEROV, 1954) *(MYXOSPORIDIA)* INFECTION IN THE SILVER CARP AND BIGHEAD

By

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(Received April 2, 1978)

The species *Myxobolus pavlovskii* had been originally described by ACHMEROV (1954) as *Disparospora pavlovskii*, in the gills of a silver carp caught from the river Amur. The genus *Disparospora* was rendered synonymous to the genus *Myxobolus* by SHULMAN in 1966. Later MUSSELIUS (1969) found the species in both silver carp *(Hypophthalmichthys mollitrix)* and bighead *(Aristichthys nobilis)* hosts originating from Asia and naturalized in Europe, and NAUMOVA and MOCHALKIN (1975) called attention to the economic importance of the parasite in pond farms. SAPOZHNIKOV and KOZACHENKO (1975) found host populations massively infected with the parasite in the Ukraine, and established elevated white blood cell counts in the diseased fish. In Hungary, SZAKOLCZAI and MOLNÁR had found the parasite already in 1966, and described it as *Myxobolus sp.*; later, MOLNÁR (1971) reported investigations into the pathogenic aspects of the species. The responsibility of *Myxobolus* parasites for losses among silver carps was also reported from China (WU et al., 1975), but in this instance the species *Myxobolus drjagini* was incriminated.

Practical and experimental observations on *M. pavlovskii* infection in pond farms, and histopathological evaluation of the disease are reported in this paper.

Materials and methods

The test material was collected in three fry-rearing pond farms situated in different regions of Hungary. The populations have been under observation since 1968, when the first outbreak of clinical illness had occurred, and experimental studies were begun in 1969.

Hosts were taken from the infected ponds at weekly or biweekly intervals for parasitological sectioning, and subsequent processing of the gills for histopathological examination. The non-infected hosts used in the experiments had been either procured from pond farms free from myxobolosis, or were hatched and reared under sterile conditions.

The laboratory experiments were conducted in aerated aquaries of 40 litre volume, and the experimental fry was fed a commercial fish diet (meat meal + maize meal) throughout.

The field experiments were either conducted in minor fry rearing ponds, or in sieve-covered crates immersed in these.

The gills removed for microscopic examination were fixed in 4% formalin and Bouin solution, and were embedded in paraffin. Longitudinal sections were stained with haematoxylin and eosin, Farkas–Mallory's stain or Giemsa stain.

Incidence of the parasites

The 2—3 cm long silver carps and bigheads, which had been imported to Hungary from natural habitats in China in 1963, had carried many *M. pavlovskii* cysts on the gills. Host populations introduced to Hungary later (from the USSR) were free from the parasite, and those pond farms in which no "Chinese" fish had been accommodated remained "clean" until the beginning of the large-scale herbivorous fish breeding programme, during which the

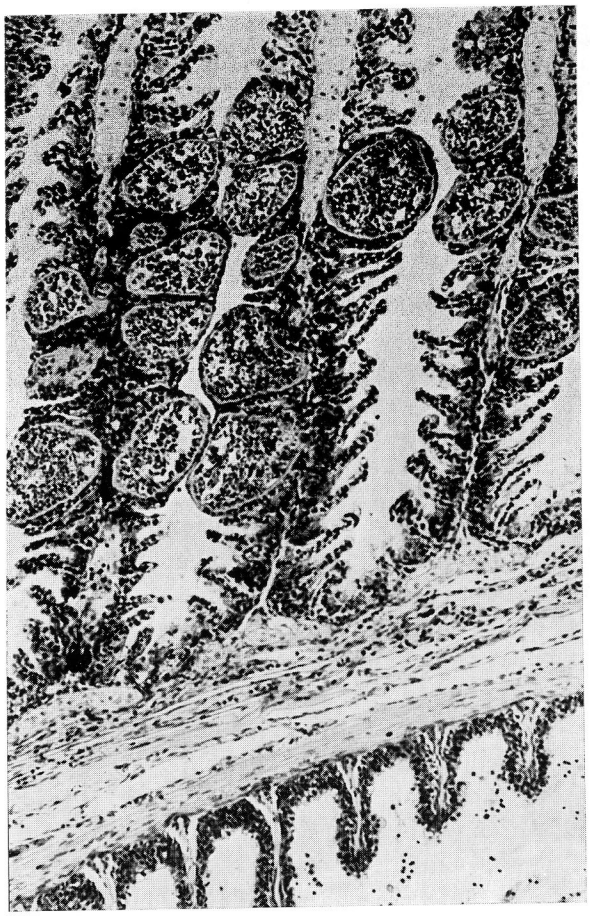

Fig. 1. Gill filaments packed with *Myxobolus pavlovskii* cysts in a silver carp. H. E., ×300

infection was disseminated on a country scale with the distribution of fry from the infected stock breeding ponds.

During the last two years, *M. pavlovskii* infection was demonstrated in all age groups of the silver carp and bighead populations; the infection was most massive among the fry, while the occurrence of cysts was rare among the more than three-summer old fish.

In the infected pond farms, appearance of cysts on the gills was noticed already in the fry-rearing ponds. At a later stage of rearing, the intensity of the infection usually showed a decreasing, less often an increasing, tendency. One-summer hosts free from *M. pavlovskii* infection were only exceptionally encountered at screening.

Developing cysts were noticed 21 days after stocking of the rearing pond at the earliest. Most cysts were localized on the gill filaments *viz.* in the stratified epithelium filling the interspaces between respiratory lamellae (Fig. 1), in case of heavy infection, however, some of the cysts developed in the epithelium of the gill rakers. The earliest stages were 8—10 μm in diameter, and were separated from the surrounding tissues by a 1.5—2.0 μm thick capsule. Cysts of this size filled the greater part of the space within lamellae, and comprised several young spores.

The growth to the cysts, and spore formation within them, lasted long. Older cysts grew more than 1 mm large. The cysts persisted in the gill tissue over several months, and massive infection was often found even after wintering. (The infection persisted in the aquary fish 6 months long).

Infection experiments

Several such experiments were carried out in each year in both pond and laboratory, to obtain further information on the developmental cycle of *M. pavlovskii.*

Attempts were made at infecting with spores hosts reared under infection-free conditions in the laboratory. The spores to be used for experimental infection had been artificially liberated from mature cysts and, depending on the actual sheme of experiment, were administered to experimental fish either immediately on release, or after storage for some days or months, or after their natural wintering in the pond. The infective spores were either given directly into mouth or mixed to the diet. All attempts were unsuccessful. Fish became infected only in a single laboratory experiment when muddy water from a pond was used as a source of infection.

In the field experiments 3—5 weeks old fry, reared free from parasite infection, always contracted the parasitosis on exposure in an infected pond within a sieve-topped crate for at least 24 hr. The intensity of infection increased with the length of exposure, but it did not differ between hosts ex-

posed in crates at the water surface or at the pond bottom. The frequency of infection was the same in the silver carp and bighead populations.

In one experiment, a group of 500 young bighead fry was placed in each of three ponds of 100 m^2 basal surface, in which outbreaks of myxobolosis had been recurrent. Pond No. 1 had been desiccated for one week, and the moist spots in its bottom had been strewn over with quicklime. Into pond No. 2 mud was introduced from the adult bighead pond on the day of the former's flooding. In pond No. 3, 10 *Myxobolus*-infected one-summer bigheads were placed among the young fry. The loss of pond water by evaporation was compensated by supply of water from the surrounding canal system. Appearance of many developing cysts 30 days after stocking the experimental ponds signified a massive *Myxobolus* infection in all three groups of fry.

Some large rearing ponds of 1600 m^2 basal surface, in which severe outbreaks of myxobolosis had occurred in several years, were renewed during winter in 1974, by removing a 30 cm thick layer of mud from the bottom. A major outbreak of myxobolosis did nevertheless occur among the rearing fry 4—5 weeks after stocking, exactly as in the previous years, except that coccidiosis, which had been concurrent previously, did not reappear.

Epizootological observations

Severe outbreaks of myxobolosis in pond farms frequently accounted for mass losses among the silver carp and bighead populations. However, a direct lethal effect of *M. pavlovskii* could not be established, as it always occurred in mixed infection with other pathogenic fish parasites, above all with the intestinal coccidium *Eimeria sinensis*, and the gill parasites *Cryptobia branchialis*, *Chilodonella cyprini* or *Trichodina sp.*

Infected fish transported to the laboratory could be relieved from external parasites by treatment in antiparasitic bath, and they got free in two weeks also of coccidia; in such cases the pathogenic effect of *M. pavlovskii* could be better judged than with a concurrent infection present. Even massively infected fish of both host species survived for several months in the aquary under good conditions of feeding and ventilation, but death invariably ensued if the parasite cysts filled at least half of the spaces between the gill lamellae.

Histopathological findings

The young trophozoites developing in the stratified epithelium between the gill lamellae, were amorphous or roundish eosinophilic bodies with sometimes 3—5 nuclei and without a capsule (Fig. 2); the earliest stages always localized near the cartilagineous supporting structure of the gill filaments (Fig. 3).

Plasmodia larger than 10 μm were enclosed by an eosinophilic capsule formed by the parasite, and were surrounded by slightly flattened cells of stratified gill epithelium (Fig. 4).

The growing cyst gradually filled the entire space within the respiratory lamellae. Maturation of the spores developing within the cyst did not take place simultaneously; fully formed spores were admixing with dividing nuclei. The nuclei of the pansporoblasts and the finished spores were irregularly scattered inside the cyst. (The degree of spore maturity could be readily assessed with the Farkas–Mallory stain, which imparted a yellow shade to mature spores, orange to semi-mature ones and blue to developing forms. The development of cyst wall could be studied with haematoxilin and eosin stain). The eosinophilic cyst wall was sharply demarcated from the surrounding epithelial cells and developing trophozoites. The trophozoite nuclei were of different sizes, ranging from 2—3 to 4 μm in diameter. The round nucleolus of each was as a rule separated by a pale halo from the chromatin-rich nuclear membrane.

In the course of cyst development the surrounding epithelial cells became extraordinarily flat, but did not disappear; they ultimately formed a net-like structure around the cyst. This structure prevented direct contact of the cyst wall with the endothelial cells of gill lamellae, or with the cartilagineous structure of the gill filaments. A similar thin epithelial coat separated the cysts also from the outside world. The surrounding epithelial cells were polygonal while the cyst was young (Fig. 4), but became flat when it grew

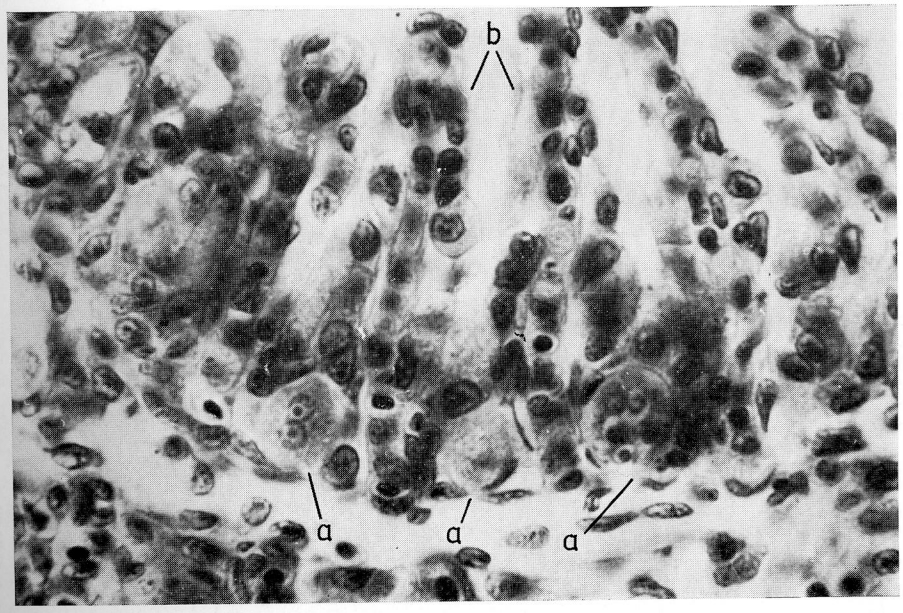

Fig. 2. Early trophozoites inside the stratified epithelium between gill lamellae. a, trophozoites; b, gill lamellae. H.E. ×1000

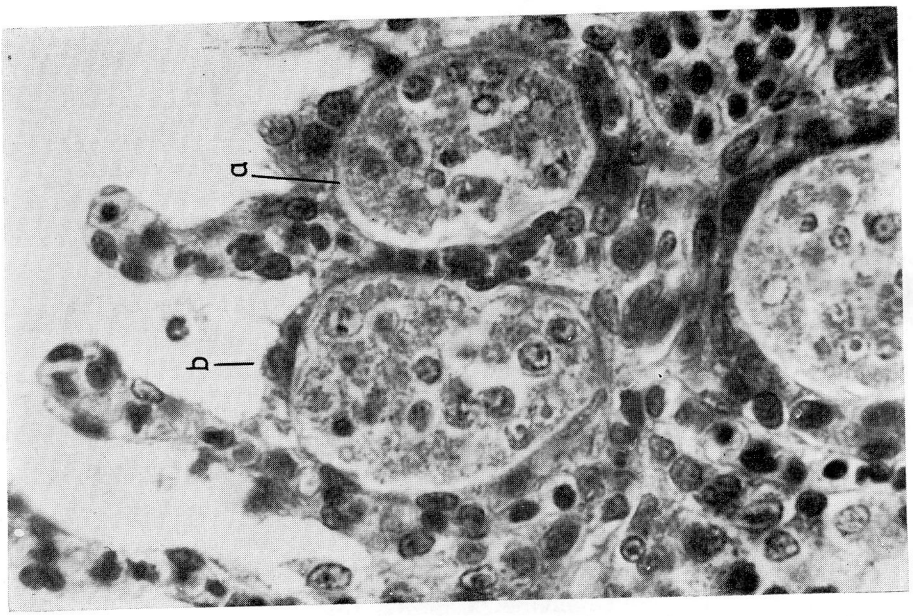

Fig. 4. Growing cysts not yet bearing spores between the gill lamellae. a, encapsulated cyst; b epithelium cell residues still showing more or less polyangular shape.

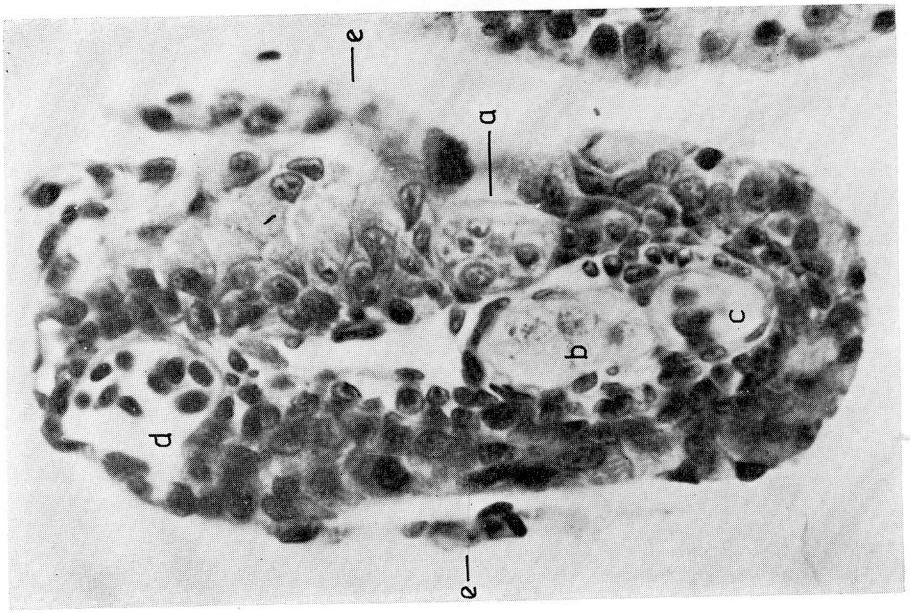

Fig. 3. Gill filament of a silver carp in transversal section. a, early trophozoite; b, cartilagineous supporting structure; c, arteria branchialis afferens; d, arteria laminae

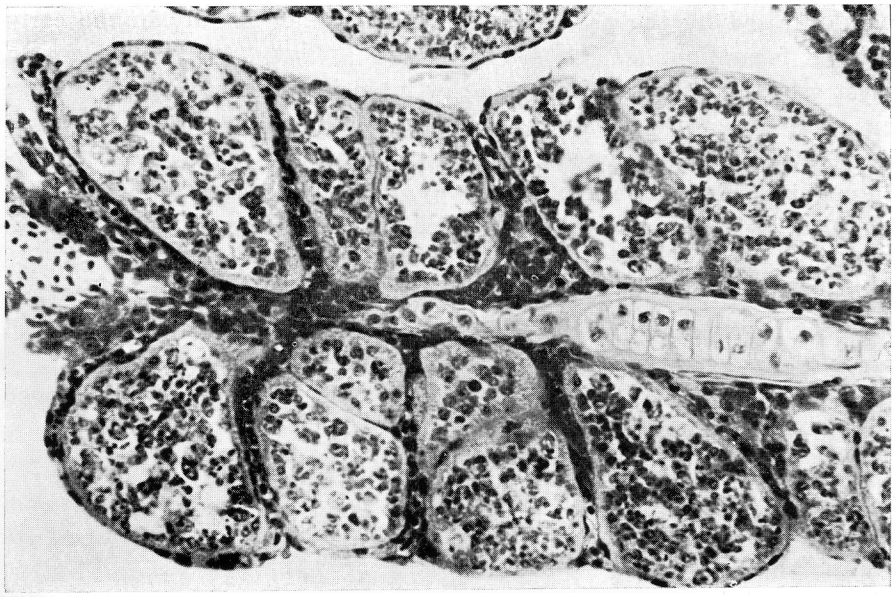

Fig. 6. Massive infection with *M. pavlovskii*. Note flattened simple epithelium around the encapsulated mature cysts. H. E. ×1000

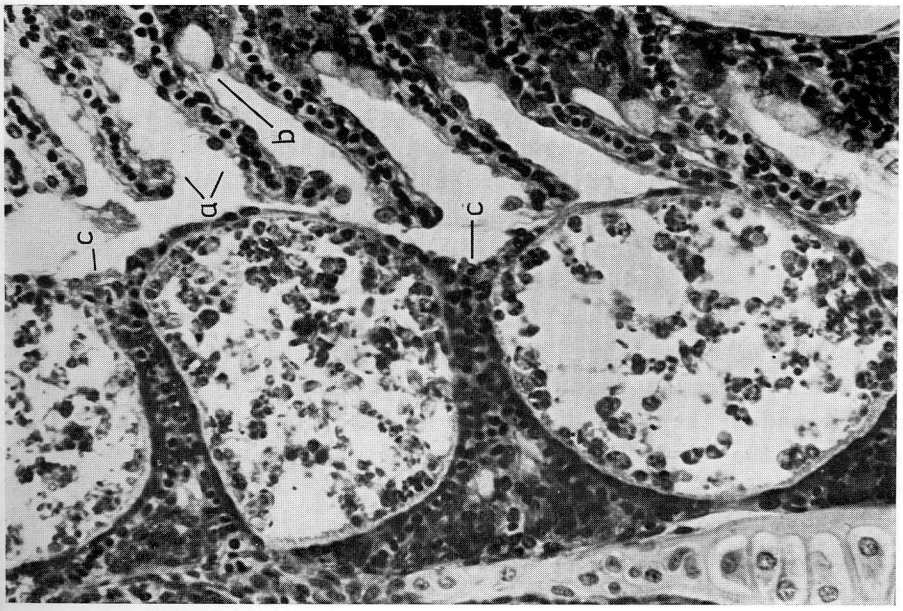

Fig. 5. Spore-bearing cysts. a, intact gill lamellae; b, goblet cells; c, gill lamellae compressed by cysts. H. E. ×1000

large enough to protrude above the level of the gill lamellae (Figs 5 and 6). Among the normal components of stratified epithelium chiefly the goblet cells were affected by the parasite; these degenerated already in the early stage of cyst formation.

Cyst growth was unaffected at a low intensity of *Myxobolus* infection; in such cases the cyst pushed the two neighbouring gill lamellae apart in opposite directions, and assumed a spherical or lenticular shape (Fig. 5). However, in massive infections, when a cyst was growing in each gill lamella interspace (Fig. 6), the developing stage sandwiched between the gill lamella planes assumed an oval shape.

If the infection was very massive, cysts also developed on the gill rakers. Development began in the raker epithelium, and the growing stages occupied the empty spaces between adjacent gill rakers.

Cellular host reaction could not be observed either the parasite developed on the gill filament or on the gill raker.

Discussion

M. pavlovskii, a common parasite of the silver carp and bighead, is very widely spread in Hungary. No unequivocal evidence of its mode of infection has been emerging from experimental studies. In ponds with a history of myxobolosis outbreak(s) of the infection reappeared among the fry in each year despite preventive measures (putting dry or freezing out of the pond); even the fish kept in floating crates near the water surface to prevent uptake of spores from the bottom mud did develop myxobolosis. The infection could be induced experimentally with pond mud among fish kept in the aquary, but oral infection with spores collected from diseased hosts invariably failed to cause disease, whether or not the spores to be used as infectious dose had been allowed to mature for several months.

Several authors (Hoffmann and Putz, 1969; Yunchis, 1974; Uspenskaya, 1978) have established that the success of experimental infection depends on adequate ageing of the spores. At the same time, Yunchis and Chernisheva (1977) have suggested that the release of amoeboid stages from the spores needs certain osmotic conditions and chemical components, which greatly depend on the host's diet. I believe that dietary factors had been responsible for the failure of my infection experiments in the aquary, for the reproduction of the natural diet of herbivorous fish is extremely difficult.

The sole responsibility of myxobolosis for fish death could not be established under natural conditions, as concurrent infections by several other protozoa were always present. Evidence was, however, obtained in the laboratory that massive infection by *M. pavlovskii* may be lethal.

GREVEN (1956) and MCRAREN et al. (1975) concluded from observations on *Myxobolus exiguus* and a *Henneguya sp.*, respectively, that cyst development began in the gill capillaries. According to my own experience, the amoeboids of *M. pavlovskii* had started development inside the stratified epithelium between the gill lamellae, and became compressed against the gill capillary walls only in a later stage of their growth.

The gill-attached cysts were separated from the outside world only by a single extremely thinned layer of host cells, but even older cysts bearing mature spores persisted on the gill filaments for a fairly long time. Spore maturation was extraordinarily irregular: mature spores were admixing with developing forms in any region inside the cyst. A similar gill parasitosis was observed by MCCRAREN et al. (1975) in catfish infected with a *Henneguya sp.;* the stages developing between gill lamellae, designated by the descriptors as intralamellar, were found to be less pathogenic than those growing between basal cells (interlamellar form), yet accounted for considerable depression of the host's resistance.

SAPOZHNIKOV and KOZACHENKO (1975) demonstrated a considerable increase in white blood cell count and gill haemorrhages in fish infected by *M. pavlovskii.*

I failed to substantiate the presence of a cellular reaction and of haemorrhages in the present histopathological studies. Although the growing cysts accounted for complete destruction of the epithelium between lamellae they did not directly injure the capillary network supplying the gill lamellae. It appears that *M. pavlovskii* has very well adapted itself to its host, which thus does not mobilize much of the protective mechanisms on infection. The primary damage of the *Myxobolus* cysts is mechanical interference with the gill's respiratory function by their bulk. Cysts adhering to the gill lamella wall prevent the contact of adjacent lamellae with the outside world, and by compressing also the still intact plates they account for reduction of the gill surface and thereby of air exchange. This accords well with the earlier observation of JACZÓ (1942) that *Myxobolus*-infected perch *(Perca fluviatilis)* hosts consumed much less oxygen than healthy ones. Destruction of goblet cells and stratified epithelium in the gill also accounts for diminution of the host's resistance to external influences on the gills. It is known that regeneration of the injured respiratory epithelium starts in the stratified epithelium, and that the increased secretion of goblet cells plays an important role in the prevention of the consequences of gill injury.

Summary

Myxobolus pavlovskii (Achmerov) is a very widely spread and important parasite of cultured silver carps *(Hypophthalmichthys mollitrix)* and bighead *(Aristichthys nobilis)* in Hungary. The cyst stages of the parasite invade above all the gills of young fish in fry-rearing ponds.

In ponds freshly stocked with fry, spread of the infection was completed in 24 hr, while experimental infection of aquary fish with orally administered *M. pavlovskii* spores failed altogether.

The most frequent localization of the myxobolus cysts is the stratified epithelium in the interspaces between neighbouring gill lamellae, but cysts may also appear on the gill rakers, if the infection is very massive.

Trophozoites begin to develop near the cartilagineous supporting structure of the gill filament and, growing larger, they destroy the stratified epithelium and goblet cells, fill the interspaces between the respiratory plates and hamper gas exchange. The thickly encapsulated cysts are separated from the outside world only by a single thinned row of residual cells from the stratified epithelium. Spore maturation inside the cyst is an uneven process. The first mature spores appear about 4 weeks after infection.

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