Epicellular coccidiosis in goldfish

Kálmán Molnár, Csaba Székely*

Institute for Veterinary Medical Research, Centre for Agricultural Research, HAS, POB 18, 1581 Budapest, Hungary

- corresponding author

ABSTRACT: In a goldfish stock held in a pet fish pond, heavy coccidian infection, caused by an epicellularly developing *Goussia* species, appeared in April of three consecutive years. The shape and size of its oocysts resemble to an inadequately described species, *Goussia carassiusaurati* (Romero-Rodrigez, 1978). In histological sections, gamogonic and sporogonic stages infested mostly the second fifth of the intestine, where almost all epithelial cells became infected. Both gamonts and young oocysts occurred intracellularly but in extracytoplasmal position, seemingly outside the cells. Oocysts were shed unsporulated. Sphaeroid to ellipsoidal unsporulated oocysts measured 12.4×13.5 µm on average, but after 48 h sporulation in tap water they reached 16×13 µm oocyst size, in which the four elliptical sporocysts of 13×5.4 µm located loosely. The size of oocysts and sporocysts are smaller than those of the better known *Goussia* species, *Goussia aurati* (Hoffman, 1965).

KEY WORDS: Coccidiosis, *Goussia*, goldfish, epicellular location, seasonal development

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INTRODUCTION

Coccidia of the *Eimeria* and *Goussia* genera are common parasites of fish inhabiting European natural waters and aquaculture farms. The genus *Goussia* was first described and separated from *Eimeria* by Labbé (1896). Epicellular development of a *Goussia* species, *G. pigra*, was first demonstrated by Léger & Bory (1932). Dyková & Lom (1981) created a new genus *Epieimeria* for epicellularly developing fish coccidia selecting *Epieimeria anguillae* (Léger et Hollande 1922) Dyková et Lom, 1981 as the type species and revitalized the genus *Goussia* Labbé. Molnár (1989) found seasonally developing coccidia of nodular and

epicellular types in cyprinid fishes and described them as *Goussia balatonica* and *G. pannonica,* respectively. Molnár (2006) regarded four genera common in fish, *Eimeria, Goussia, Crystalospora, Calyptospora* as members of the Eimeriidae family. Several authors among them Upton (2012) and Duszynski et al. (2016) still regard *Goussia* as synonym of *Eimeria*. In recent papers (Molnár et al. 2012, Rosenthal et al. 2016) where molecular sequences of different fish coccidia were examined, it was, however documented that great differences exist among *Eimeria* and *Goussia* spp, more over within the members of *Goussia* at least four groups can clearly be differentiated. Among *Goussia* spp. collected from Hungarian fishes Rosenthal et al. (2016) found that molecular sequences of species with small oocysts developing dispersedly in the gut of their hosts throughout the year differ from sequences of species with large oocysts developing seasonally in nodules and epicellular sites. Great differences were also found between nodular and epicellular species. Species infecting the kidney composed the fourth group.

Four coccidian species have been reported from the gut of goldfish. *Goussia aurati* was described by Hoffman (1965) as *Eimeria aurati* from the gut of a goldfish shedding fecal casts with non- sporulated oocysts, which, once sporulated, measured 16-24 × 14-17 μm and containing elliptical sporocysts measuring 11-13 × 6.5-8 μm. He found this species similar to *Goussia subepithelialis* (Moroff & Fiebiger, 1906), the nodular coccidium of the common carp, which according to data given by Steinhagen et al. (1990) have similar sizes with round 17-19 μm oocysts and 12-14 × 3 μm sporocysts. Romero-Rodriguez (1978) found a similar species, *Goussia carassiusaurati* (described as *Eimeria*) in goldfish with 15.2 × 13.3 μm oocysts and 13.6 × 5.8 μm sporocysts. The third species *Goussia hupehensis* described by Chen & Hsieh (1964) as *Eimeria hupehensis* is a typical dispersed coccidium resembling closely in its morphology *Goussia carpelli* (Léger et Stankovitch, 1921). *G. hupehensis* (Chen & Hsieh, 1964) cit. by Chen & Li (1973) was found also in Hungary by Molnár et al. (2005) who experimentally proved that this species (named only as *Goussia* sp.) cannot be transmitted to the common carp. A fourth species, described as *Eimeria newchongensis* by Chen (1984) from the gut of the goldfish and *Culter erythropterus* in China, by its 17.3-20 μm sporulated oocysts, may not differ from *G. auratii*.

Here, we describe a coccidiosis in the gut of goldfish caused by epicellularly developing coccidian stages.

MATERIALS AND METHODS.

Goldfish (*Carassius auratus gibelio* (L) are frequently cultured in small hobby ponds in Hungary, as well as in aquaria. From one of these ponds where about 100 specimens of 1 to 5 years old goldfish and their about 1000 offspring was cultured some fish were sent to laboratory for health control at about in monthly intervals. Fish were collected from the pond by a hand net, carried to the laboratory alive in oxygenated plastic bags, kept in aerated aquaria. They were sedated with 20 ppm clove oil added to the water and exterminated by cutting their head. Coccidian infection of varying intensity with small sized oocysts of the dispersed *G. hupehensis* species was recorded throughout the examination period. In addition in Aprils of three consecutive years heavy infection with large sized oocysts resembling epicellular and nodular coccidia, common at this time in natural water fishes (Molnár 1989), were recorded. In April, 2016 a specific survey for coccidian infections was performed on twenty five specimens of 1 to 3 years old goldfish specimens measuring 4 to 13 cm. The gut was opened lengthwise under a dissecting microscope and cut into five equal parts. From the epithelial surface, pieces of clean mucus were removed, placed under coverslip, and studied with a Nomarski differential interference contrast with an Olympus BH2 microscope. Some non-sporulated oocysts were placed in tap water into a small Petri dish and left to sporulate for 48 hours. Histological sections were prepared in 2016. In April 5, 15, and 25 the gut of the smallest fish, about 4 cm in length, was separated and coiled into a roll, fixed in Bouin's solution for four hours, embedded in paraffin wax, cut to 4-5 μm sections, and stained by haematoxylin and eosin. Non-sporulated and sporulated oocysts were studied using Nomarski differential interference contrast with an Olympus BH2 microscope. The oocysts and histological sections were photographed with an Olympus DP 20 digital camera. From oocysts digitised images were obtained and measurements were taken with the IMAGO® software.

RESULTS

Heavy infections with non-sporulated oocysts were found in April in each year. At this time the gut of these fish was empty or contained only very little faeces. The fish were in poor condition but no mortality was recorded. No mortality was recorded on such fish subsequently held in laboratory aquarium for about a month. All of the examined fish proved to be infected. When freshly dissected, the second and fifth sections of the intestine were found to be most heavily infected with gamogonic and sporogonic stages. Relatively little infection was recovered in the first and third sections of the gut. From the last two sections, only non-sporulated oocysts were recovered and these were located free in the lumen of the gut or in faces. Oocysts were shed unsporulated. Most of the unporulated oocysts had a granulated structure and spheroid or short ellipsoidal shape (Fig. 1). In the first half of April, mostly developing gamonts were found in the first part of the intestine (Fig. 2). In the middle of April only non-sporulated oocysts were found in infected sections of the gut. By the end of April most oocysts had left the gut and only sparse oocysts were found in the rectum among faces. In tap water at 22-23 °C, non-sporulated oocysts became sporulated in two days. Sporulated oocysts had also spheroid or short ellipsoidal shape (Fig. 3), a very thin, single layered and colourless wall. The oocysts (Table 1) measured 12.4±0.86 (12.8-15.2) μm in length and 13.5±1.8 (12-14.6) μm in width. There was no oocyst residuum, but a small polar body existed in oocysts. The four thin-walled, elongated ellipsoidal sporocysts located loosely in the oocysts, measured 13±0.8 (11.2-15.4) μm in length and 5.4±0.34 (4.5-5.6) μm in width. In each sporocyst there were two vermiform sporozoites arranged head to tail. Sporozoites measured 10.3±0.73 (9.6-11.2) μm in length and 2.5±0.32 (2.4-2.8) μm in width. In freshly sporulated sporocysts, the sporocyst residuum covered most parts of the sporozoites, after 48 hours sporulation, this residuum became short and ellipsoidal, an about 2.5 × 1.3 compact body, and it located in the sporocysts among sporozoites.

In histological preparations fixed in the first half of April, heavy infection with young gamogonic stages was found in the second and third fifths of the intestine, while developing

oocysts were only rarely found in the first fifth, and they were not found in the fourth and fifth segments of the gut. At this time, most of the gamonts were young, measuring about five μm in diameter. In some segments of the intestine, they infected almost every epithelial cell (Fig. 4). Less frequently, microgamonts were also found (Fig. 5). Development of gamogonic and sporogonic stages was not synchronous, as mature, unsporulated oocysts were found among developing gamonts (Fig. 6).

DISCUSSION

Although the genus *Goussia* is still regarded by many authors synonym to *Eimeria* (Upton 1912, Duszynski et al. 2016), others working on eimerians of fish and anurans accept its validity (Lukeš & Dyková 1990, Steinhagen et al. 1990, Lukeš 1992, Jirkú et al. 2002, 2009, Dogga et al. 2015). Recent phylogenetic works (Molnár et al. 2012, Rosenthal et al. 2016) have validated deep evolutionary distinctions among the coccidian parasites of fish, including (but not limited to) a monophyletic assemblage limited to true members of the genus *Eimeria;* subdivisions within *Goussia* correspond to morphological and developmental attributes that likely warrant further taxonomic revision. Several *Goussia* species develop in nodules in the gut like *G. subepithelialis*, while others develop in epicellular position in the enterocytes like *G. pigra.* Molnár (1989) who described *G. balatonica* and *G. pannonica* spp. from the white bream, remarked that in several cyprinids a mixed infection with nodular and epicellular type coccidia occur in the time of oocyst formation in April. Morphologically similar oocysts which, however, differ in size also occur in non-cyprinids; these include *Goussia aculeati*, and *G. zarnowskii* in *Gasterosteus aculeatus* (L.) and *G. acipenseris* and *G. vargai* in *Acipenser ruthenus* (L.) (Jastrzebski 1984, Molnár 1986). It is not excluded therefore, that epicellular and nodular coccidia exist concurrently also in the common carp and the goldfish. Both fish species is of Chinese origin which introduced only a smaller part of their parasite fauna to Europe. The smaller (in average 16 μm) oocyst size measured for samples of the goldfish in the present investigation resembles to epicellular *G. pannonica* studied by Molnár (1989), and *G. janae* described by Lukeš & Dyková (1990), while *G. aurati*, with its 16×20 μm large oocysts described by Hoffman (1965), correspond better to a nodular species. Although the above facts might suggest the nodular nature of *G. aurati* and the epicellular nature of *G. carassiusaurati* present data give not enough basis for drawing this conclusion.

Similar problems exist at the common carp. To date, only a nodular species, *G. subepithelialis*, has been reported from this fish. It cannot be ruled out, however, that a more detailed examination in China, in the original biotope of the common carp and the gold fish both nodular and epicellular species could be recorded.

Epicellular development characterizes a particular lineage of coccidia, which adds weight to the validity of the genus *Epieimeria* Dyková & Lom, 1981. On the other hand, the great difference in morphology, development and molecular sequences between *Goussia* type epicellularly developing coccidia and the type specimen *Epieimeria anguillae* contradicts a relationship between the two eimerian groups. Differences in the development (permanently developing group vice versa annually developing groups), intracellular location (intracytoplasmal and epicytoplasmal), more over differences in the molecular sequences among dispersed, nodularly and epicellularly developing groups (Rosenthal et al. 2016) would favour creation of new coccidian genera in the future, among them a genus with a tentative name *Epigoussia*.

Acknowledgements The authors thank G. Pataki for the preparation of histological slides. The authors are also thankful to dr. Yang Liu (College of Fisheries, Huazhong University, Wuhan, China) who helped the authors to the Chinese separates. Dr. Benjamin Rosenthal's suggestions and help in correcting the English of the manuscript are also greatly appreciated.

This study was financially supported by the by the Hungarian Scientific Research Fund (OTKA, project No. 100132) and by GINOP 2-3-2-15-2016-00004 project.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed. Permit for scientific fishing in Hungary (EHVF/121-1/ 2014) is issued by the Ministry of Agriculture, Hungary.

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List of figures:

Fig. 1. Non-sporulated *Goussia* oocysts shead by a goldfish. Bar=10 µm

Fig. 2. Young gamogonic *Goussia* stages in a scraping of the intestinal epithelium. Bar=20 µm

Fig. 3. Sporulated *Goussia* oocysts with round sporocysts and sporozoites (arrows). Bar=10 µm

Fig. 4. Histological section of the second fifth segment of the gut. Young *Goussia* gamonts in epicellular position (arrows). Haematoxylin and eosin staining. Bar=20 µm

Fig. 5. Micro- (arrows) and macro- *Goussia* gamonts (open arrows) locating epicellularly in the intestine of a goldfish. Haematoxylin and eosin staining. Bar=10 µm

Fig. 6. Asynchronous *Goussia* development in the intestine of the goldfish. Oocysts (open arrow) and young gamogonic stages (arrows) occur in the same time. Haematoxylin and eosin staining. Bar=20 µm