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Comments on the Nature and Methods of Collection of Fish Coccidia

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ABSTRACT. Eimerian species of fish are little studied owing to the difficulties in their recovery and storage. Common separation procedures usually injure the extremely thin and vulnerable wall of the oocyst. For the detection of intestinal fish coccidia native investigation of the intestinal content (faeces), intestinal mucus and mucosal scrapings is recommended, while the species localizing in the tissues should be separated by digestion with 0.25-0.5% trypsin solution. Stable microscopic preparations can be made from samples of faeces, mucus or tissues spread on a slide by compression with a coverslip and by the instillation of 4% formaline or 2.5% glutaraldehyde and sealed with Canada balsam.

Up to now about 100 Eimeria species have been described from fish, above all from freshwater hosts. This is a very low number compared to the number of coccidian species known in warm-blooded animals. PELLÉRDY's monograph (1974) includes the description of 75 fish coccidia, since then about two dozen new species were described mostly by the present writer and his coworkers (MOLNÁR and FERNANDO, 1974; MOLNÁR and HANEK, 1974). Coccidia are only infrequently dealt with in papers on the incidence and dynamics of fish parasites, although according to our own experiences, Eimeria species can be collected from fish hosts relatively easily by aimed investigation. Since the main reason of the apparently limited interest in Eimerian parasites of fish seems to be the inadequacy of methodical approach, some aspects facilitating the study of fish coccidia will be outlined in this paper.

Main characteristics of fish coccidia

Eimerian parasites of fish differ from those of mammals and birds in certain morphological and developmental aspects, but attempts for classification of the genus Eimeria, including the segregation of fish Eimeriae (LABBE, 1893; PELLÉRDY, 1964) have failed for lack of feasible differentiating features. Thus in the following description of those features of fish Eimeriae will be given, which facilitate the detection and collection of these parasites.

1. Most Eimeria species living in fish are passed by the host in a sporulated state. This is a fairly general rule with fish coccidia, although there are some exceptions, such as Eimeria pigra and E. aurati, which undergo sporulation outside the host, or E. micropteri, most oocysts of which become extruded in a semi-sporulated state.

2. Since abrupt environmental changes are not common in the aqueous habitat, the coccidia of fish are less resistant to external influences than those of other host species. Low resistance is obviously due to the thinness of the shell of both oocyst and sporocyst. The approx. 30 species studied by the present writer all had an unilayered wall.[x] The thickness of the

[x] In the drawings presented with related publications (PELLÉRDY and MOLNÁR, 1968; MOLNÁR and PELLÉRDY, 1970) and in PELLÉRDY's monograph (1974) as well, the depiction of the oocyst wall is faulty, showing it to be thicker than it is in reality, although the description of it is correct.

oocyst wall did not exceed 130 Å in the species studied by LOM (1971). The thin wall is extremely sensitive to exsiccation as well as mechanical and osmotic effects, and is liable to injury. The sporocyst wall is bilayered, structurally much more stable, and also more resistant; according to LOM (1971) the wall of the E. subepithelialis sporocyst consists of a 200 Å thick outer layer and a 700 Å thick inner one. The sporocyst wall may also be quite thick in certain fish coccidium species, e.g. in E. siliculiformis.

3. It is remarkable that several known species of fish coccidia (E. gadi, E.metschnikovi, E. siliculiformis, etc.) establish themselves in organs (air bladder, liver, spleen, serous membranes) from which they cannot pass to the outside unless the host dies.

4. Most fish coccidium species are characterized by a double-shelled, sutured spore, which had once served as one differentiating feature of the obsolete genus Goussia. However, in several species (E. moronei, E. salvelini, E. anguillae, E. truttae) either a typical Stieda body, or a terminal cap has been recognized at the more tapered end of the sporocyst.

It follows from the foregoing characteristics that the coccidia of fish generally differ from those of other animal species. However, these differences are neither consistent, nor regular, Fish coccidium species displaying all general features of Eimeriae may exceptionally be found. The main differentiating feature from the coccidia of warm-blooded animals is the extremely thin oocyst wall, which can among others account for the rare detection of such parasites. Procedures employed for the demonstration of mammalian or avian coccidia (flotation, sedimentation, incubation in potassium bichromate solution) are less applicable with fish coccidia, owing to the vulnerability of the thin oocyst wall. Immobile protozoa usually escape the attention of the fish pathologist not versed in coccidiology, large their number may be.

The technics of oocysts recovery

The technical approach depends on the localization of the parasite. With intestinal Eimeriae the examination of unstained smears of faeces, mucus and mucosal scrapings proved to be the method of choice, whereas with those localizing in tissues, the microscopic examination of impression smears has been most succesful.

Examination of the faeces: Only the experienced examiner can detect oocysts in the faeces, and only in the case of massive infection. Faecal samples taken from different segments of the intestine are transferred to the slide and pressed by a coverslip for microscopic examination. Glossy sporulated oocysts become visible at 400 fold magnification.

Examination of intestinal mucus: The intestinal contents of fish are often surrounded by a deposition of mucus along the epithelial surface of the mucosa. This deposition is nearly always found in hosts massively infected by coccidia. With some experience the mucous deposit can easly be removed from the solid intestinal content, even from the passed faeces, and it can be secured without adherent clumps of faeces if the fish have been starved for one or two days. The mucous deposit, colourless or yellowish, can easily be examined for oocysts under a coverslip. The oocysts are frequently embedded in a so-called yellow body, and occur either singly or in groups of 2 to 5. If the intestinal content is covered by too small amount of mucous deposit, mucus can be collected from the surface of the intestinal epithelium by cautious scraping. In this case many epithelial cells become admixed with the sample interfereing with the detection of the oocysts.

Examination of mucosal scrapings: This approach is less reliable than the other two, because part of the oocysts localizing in the intestinal mucosa are still in the unsporulated state sendering their detection more difficult. Parallel microscopic examination of the faecal mucous deposit and mucosal scrapings is nevertheless strongly recommended, because Eimerian species establishing themselves in foci or in deeper layers of the propria such as E. subepithelialis can still be most easily found in this manner.

Flotation, filtration and sedimentation: Coccidian oocysts of warm-blooded animals are separated from the faeces chiefly by flotation, occasionally combined with other procedures

(homogenisation, filtration, sporulation, aeration, etc.) to increase the efficiency of recovery. The optimal procedure has been described in detail by RYLEY et al. (1976). These methods are generally impracticable with fish coccidia. Flotation usually gives rise to deformation of the oocysts, and is therefore out of question. Filtration of the oocysts through a fine mesh and subsequent sedimentation is a valuable complementary procedure to faecal examination.

Sedimentation is a relatively rapid procedure, oocysts can be recovered from the sediment, but although the soluble and coarse fibrous components are removed by previous processing, masses of faecal debris are present in the sediment together with oocysts.

Digestion: An oocyst population free of debris can be obtained exclusively by digestion. The mucosal lining of the infected segment of intestine is scraped off after through washing, and is placed in an 0.25-0.5% trypsin solution. Trypsin can be changed repeatedly by cautious centrifugation and decantation of the supernatant. Tissue scraps gradually become digested until finally only oocysts are found at the bottom of the centrifuge tube. The oocysts so collected can be stored in water for a relatively long time. (In our experiments trypsin did not injured the oocysts, nor the sporocysts released from disrupted fully sporulated oocysts, not even when bile or 0.75% desoxycholate was added to the system.)

Examination for oocysts localizing in tissues is made as follows: a small piece of tissue, the size of a pea, is impressed between two slides, or between two glass plates if is larger, and examined under the microscope. Since such oocysts usually form groups, the aggregations may be recognized already at a low power of magnification. With small fish hosts the entire gut can in toto be examined in this manner. With larger organs the digestion procedure should be employed, as described above.

Storage of oocysts

Oocysts purified by digestion or sedimentation could be stored in water over periods ranging from two weeks to five months without notable morphological change. Oocysts recovered from the faeces (E. carpelli, E. sinensis) soon became disrupted in the course of storage, however, the contours of sporocysts and residual body could distinctly be recognized inside the resistant sporocysts for several months.

Oocysts deteriorated rapidly in 4% formaline or 2% potassium bichromate solution, but addition of a few drops of 4% formaline to the faecal sample did not affect them. In view of this, a practicable method was elaborated for use in field studies. Samples of mucus were taken from the gut of the examined fish host, preferentially from the anterior intestine. The host had been starved for one or two preceding days whenever possible, to ensure complete evacuation of the gut. If there was no other choice faeces was used instead of intestinal mucus. A small amount of mucus or faeces was transferred to a slide, not more than sufficient to cover three quarters of a coverslip, surface when compressed. A few drops of 4% formaline or 2.5% glutaraldehyde solution were subsequently instilled under the coverslip, which was then fixed in position with Canada balsam. The shape and structure of the oocysts remained preserved in such preparations for periods ranging from 4 weeks to 1 year, in the worst case for a few days at least, and the preparations were thus available for microscopic study (depiction and measurements) in the laboratory, after the conclusion of sampling in the field. The same procedure can also be used for obtaining preparations of Eimeriae localizing in tissues.

Discussion

This paper was written with the aim to encourage studies of fish coccidia by methodical instructions derived from the present writer's own experience.

According to LOM (1970) up to now about 40 species of Eimerian parasites have been described from marine fish hosts. Of some 60 Eimerian species known from freshwater fishes most

were found in Europe (LÉGER and STANKOVITSH, 1921; LÉGER and BORY, 1932). Detailed data have also been reported on coccidia indigenous in fish populations of Siberian and Far-Eastern waters (SCHULMAN and ZAIKA, 1962; CHEN, 1956). In Canada the present writer and his coworkers (MOLNÁR and FERNANDO, 1974; MOLNÁR and HANEK, 1974) demonstrated a considerable number of fish coccidium species. In other parts of the world fish coccidia have been little studied, if at all; no data are available on their occurrence in Africa, South America and Australia.

On studying the literature, one has the impression that the fish coccidium line has been pursued with success by only those few specialists or research teams which employed an adequate method of collection, but did not judge it as worth of description, probably because of its simplicity. At the same time, failure of attempts at separation of oocysts may have led also recognized fish pathologists to the conclusion that no coccidian parasites occur in fish hosts in the region studied. In Canada, where no fish coccidia had previously been kown, 21 Eimeria species were found in fishes and 24 of the 29 host species studied were found infested by coccidia a few years ago, when the methods described in this paper were employed for aimed investigation (MOLNÁR and FERNANDO, 1974; MOLNÁR and HANEK, 1974).

MOLNÁR, K.: A halcoccidiumok természetéről és gyűjtési módjáról

A halélősködő eimeriák kevésbé tanulmányozott volta gyűjtésük és tárolásuk nehézségeivel magyarázható. Rendkívül vékony burkú és sérülékeny oocystáik az általánosan elterjedt szeparálási eljárások során károsodnak. Kimutatásukra bélsár, bélnyálka, nyálkahártya-kaparék vagy szervelnyomatok natív vizsgálatán kívül a szövetekben lévő oocysták mesterséges emésztéssel (0,25 - 0,5%-os tripszin oldattal) való kiszabadítása ajánlható. A bélsárban, nyálkában vagy szövetben levő oocysták fedőlemez alatt 4%-os formalinnal vagy 2,5%-os glutáraldehiddel körülfolyatva és a fedőlemez kanadabalzsammal való szegélyezése után mikroszkópos vizsgálatra alkalmas állapotban hosszabb ideig tárolhatók.

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