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OCCURRENCE OF TWO NEW *GOUSSIA* SPECIES IN THE INTESTINE OF THE STERLET *(ACIPENSER RUTHENUS)*

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Two new *Goussia* species, *G. vargai* and *G. acipenseris*, are reported from the intestine of sterlets *(Acipenser ruthenus)* caught in the River Danube. The oocysts of both parasites are exreted in unsporulated state; in tap water at 20 °C they sporulate within 48 h. By histological methods the oocysts and developmental stages of the parasites can be demonstrated from the mucosal epithelium of the pyloric appendages and small intestine. *G. vargai* develops in a usual location, i.e. in the cytoplasm of epithelial cells, whereas *G. acipenseris* can be found in epiplasmal location, under the cell membrane of epithelial cells, protruding into the intestial lumen.

Keywords. *Goussia vargai, G. acipenseris*, coccidium, sterlet *(Acipenser ruthenus)*, intestine, development, location.

In recent years the demonstration of fish-parasitic coccidia has become successful. In 1983, Dyková and Lom reported 127 species belonging to the genera *Cryptosporidium*, *Crystallospora*, *Eimeria*, *Epieimeria*, and *Goussia;* since then their number has increased by further 10 species described by Li and Desser (1985*a*, *b*), Landsberg and Paperna (1985) and Jastrzebski (1982, 1985). In spite of this, no coccidia have been known to occur in species belonging to the Acipenseridae family.

In the present paper, two new *Goussia* species are reported from the intestine of the sterlet *(Acipenser ruthenus)*.

Materials and methods

The sterlets examined were mature, several years old fish that had been caught from the reach of the Danube near Paks (Hungary) and transported, for propagation, to a fry-hatching pond farm early in May. In 1984 and 1985, 16 and 24, respectively, of the sterlets were subjected to parasitological examination; their intestines were examined for the presence of coccidia. The intestines of the fish, kept on nets or in basins for a few days, contained no remnants of feed. The mucus covering the intestinal epithelium could be lifted off easily. The intestine was always divided into three parts; the first contained the glandular body including the pyloric appendages, the second the foregut, whereas the third the hindgut.

The mucus and mucosal scrapings taken from the different parts of the intestine were examined under the microscope, and the oocysts found in them were made to sporulate in tap water, in small petri dishes, together with the mucus. Bacterial growth was prevented by adding penicillin and streptomycin.

The intestines of some of the fish were processed histologically as well; the intestinal portions were fixed in Bouin's solution, embedded in paraffin, and the sections were stained with haematoxylin and eosin.

Results

Fourteen of the 16 fish examined in 1984 and 21 of the 24 ones examined in 1985 had coccidium oocysts in their intestine. In the mucus taken from the intestinal wall, unsporulated oocysts of two different sizes and round shape were found. The oocysts were of granular structure and had a uniformly dark appearance (Fig. 1). The smaller oocysts were 7 to 8 μm, while the larger ones 11 to 14.5 μm, in size. In tap water, at 20 °C, sporulation was completed within 24 h, the oocysts increased in size and transformed into *Goussia*-type coccidia. After 48 h the sporocyst residuum completely filled the sporocysts and after 72 h it became compact. Based upon their size and characteristic location determined by histological methods, the oocysts proved to belong to two hitherto unknown *Goussia* species, whose description is as follows.

Goussia vargai n. sp. (Fig. 2)

(The parasite was named in honour of Dr. István Varga, the noted Hungarian coccidiologist.)

Host and locality: *Acipenser ruthenus*, River Danube, around Paks (Hungary).

Location: mucous membrane of the intestine and pyloric appendage.

Hosts/infection: 40/35.

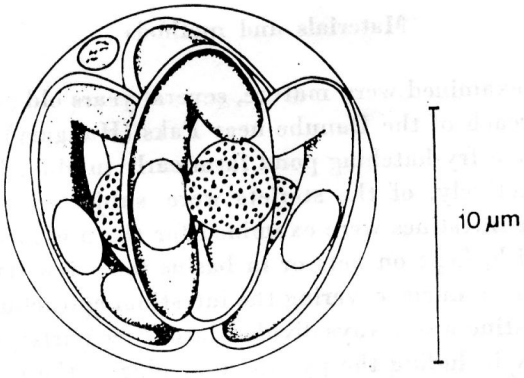


Fig. 2. G. vargai oocyst

Description (on the basis of 25 oocysts examined): The oocysts are spherical or short-ellipsoidal in shape. In sporulated state, the spherical oocysts are 12.6 to 20 μm (average: 15.88 $\pm$ 1.84), whereas the short-ellipsoidal ones 15–20 $\times$ 14–19 μm (average: 18.06 $\pm$ 1.3 $\times$ 16.38 $\pm$ 0.98) in diameter. The oocysts have a thin, colourless, single-layered wall; within them the sporocysts show a relatively loose arrangement. There is neither oocyst residue nor micropyle, but there exist one or two amorphous polar granules 1 to 1.5 μm in size. The sporocysts are elongated ellipsoidal in shape (Fig. 3) and 10.5 to 14 $\times$ 4.5 to 6 μm (11.6 $\pm$ 0.64 $\times$ 5.1 $\pm$ 0.76) in size. The sporocysts are composed of two hemispheres and have a very thin wall; in the oocyst they are arranged irregularly but mostly in one direction. In the sporocyst there are two vermiform sporozoites arranged in head to tail presentation, with one end reflexed. Without the reflexed end, the sporozoites are 9 to 11.5 μm (10.4 $\pm$ 1.69) in length and 1.7 to 2.2 μm (1.85 $\pm$ 0.09) in thickness. In the sporozoites there is a large refractile globule. After 72-h sporulation the sporocyst residue is 3 $\times$ 4 μm in size, compact, and short-ellipsoidal in shape.

The oocyst is excreted from the fish in unsporulated state. The intestine of severely infected fish contains merozoites 9.5 $\times$ 2.2 μm in size.

Histological studies. The developing oocyst and early developmental stages of *G. vargai* were found in the epithelial cells of pyloric sacs of the glandular body and in those of the foregut mucosa. The meronts (Fig. 4), each containing 16 merozoites 11 $\times$ 2 μm in size, were located in the cytoplasm of the epithelial cells, and varied between 9–11 $\times$ 11–13 μm in size. On one occassion I found a subepithelially-located meront 23 $\times$ 17 μm in size; it contained 40 merozoites 8 $\times$ 2 μm in size (Fig. 5). The macrogamonts (Fig. 6), sized 7–13 $\times$ 12–17 μm, and the microgamonts (Fig. 7) 10–13 $\times$ 15–17 μm in size were mostly in the cytoplasm between the nucleus of epithelial cells and the lumen; occasionally, however, they were demonstrable basal to the nucleus (Fig. 8). The degree of infection varied between fish, but within a given fish there was no difference

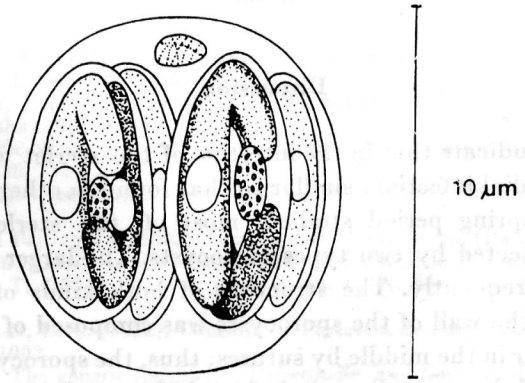


Fig. 9. G. acipenseris oocyst

between the number of parasites present in the foregut and in the pyloric sac.

Goussia acipenseris n. sp. (Fig. 9)

Host and locality: *Acipenser ruthenus*, River Danube, around Paks (Hungary).

Location: mucous membrane of the intestine and pyloric appendage; the part of epithelial cells immediately under the surface.

Hosts/infection: 40/28.

Description (on the basis of 25 oocysts examined): the oocysts (Fig. 3) are short-ellipsoidal in shape and 9.6–10.7 × 7.5–9.7 μm (average: 10.17 ± ± 0.54 × 8.47 ± 0.55) in size; their wall is very thin, single-layered and colourless. The sporocysts show rather compact, usually unilateral, arrangement within the oocysts. There is neither oocyst residue nor micropyle, but there exists a polar granule 0.5 to 1.0 μm in size. The sporocysts are elongated ellipsoidal and 6.6–8.8 × 3.0–4.3 μm (average: 8.11 ± 0.83 × 3.4 ± 0.36) in size. The sporocyst consists of two hemispheres and has a very thin wall. In the sporocyst there are two vermiform sporozoites arranged in head-to-tail presentation with one end reflexed. Without the reflexed end, the length of the sporozoites is 7.2 to 8 μm (average: 7.72 ± 0.17), while their thickness is 1.1 to 1.6 (1.31 to 0.18) μm. The sporozoites contain a large refractile globule. After 72-h sporulation the short-ellipsoidal sporocyst residue is 2.5 × 1.3 μm in size.

The parasite is excreted from the fish in the form of unsporulated oocysts.

Histological examination. Both the developing oocysts and the early developmental stages of *G. acipenseris* can be found in characteristic location, i.e. in epiplasmal position, under the luminal cell membrane of the mucosal epithelial cells lining the glandular body's pyloric sacs and the intestine, and protruding from the cells (Fig. 10). The 4–6 × 4.5–7 μm-sized meronts (Fig. 11) contain 8 merozoites 3 × 2 μm in size. The macrogamonts (Fig. 12) are 6.5–8 × 8.9, while the microgamonts (Fig. 13) 4–5.5 × 6.5–7.5 μm in size.

Discussion

The results indicate that in the intestine of the sterlet and other acipenserids severe coccidial infection, similar to that found in other fish species, may develop. In the spring period studied, most of the sterlets prepared for spawning were infected by two types of oocysts. The larger oocysts occurred somewhat more frequently. The structure of both kinds of oocysts was of *Goussia*-type, i.e. the wall of the sporocysts was composed of two equal halves joining one another in the middle by sutures; thus, the sporocysts were morphologically typical of the genus *Goussia* Labbé, 1896, revalidated by Dyková and

Lom (1981). On the other hand, the histological examinations revealed fundamental differences in the development of the two species. *G. vargai*, like the majority of species belonging to the Eimeriidae family, developed in the cytoplasm of epithelial cells of the intestine and pyloric appendages, whereas *G. acipenseris* occupied an epiplasmal position in the very same epithelial cells. Development similar to that of the latter species was first reported by Léger and Hollande (1922) and Léger and Bory (1932), who thought that the eel parasite *Eimeria anguillae* and *E. pigra* parasitizing the rudd developed extracellularly, attached to the surface of the epithelium. Dyková and Lom (1981) took into account the same postulated epicellular development when they established the genus *Epieimeria*, into which, however, they were unable to assign *Goussia (Eimeria) pigra*, a species having Goussia-type oocysts. Recently, using electron-microscopic methods, Molnár and Baska (1986) have demonstrated that *Epieimeria anguillae* does not have extracellular merogony and gamogony as these stages develop within the epithelial cell but in a parasitophorous vacuole bordered solely by the cell membrane. In the present case no electron-microscopic studies have been performed; however, it still seems doubtless that *G. acipenseris* has a mode of development similar to that of *E. anguillae*. Since *G. pigra* and *G. acipenseris* cannot be classified into the genus *Epieimeria*, the justification of attempts made by Dyková and Lom (1981) and Levine (1983, 1984) at dividing the genus *Eimeria*, formerly considered to be an integral whole, is questionable. At the same time, our studies, together with the results of Landberg and Paperna (1985) and Jastrzebski (personal communication) call attention to the fact that the epiplasmic mode of development and excretion of oocysts from the fish intestine in unsporulated state may be much more frequent than indicated by data of the contemporary special literature.

References

Dyková, I. and Lom, J. (1981): Fish coccidia: critical notes on life cycles, classification and pathogenicity. J. Fish Dis. **4,** 487–505.

Dyková, I. and Lom, J. (1983): Fish coccidia: An annotated list of described species. Folia Parasitol. (Praha) **30,** 193–208.

Jastrzebski, M. (1982): New species of intestinal coccidia in freshwater fish. Bull. Acad. Pol. Sci. Ser. Biol. **30,** 7–11.

Jastrzebski, M. (1984): Coccidiofauna of cultured and feral fishes in fish farms. Wiad. Paras. **30,** 141–163.

Landsberg, J. H. and Paperna, I. (1985): *Goussia cichlidarum* n. sp. (Barrouxiidae, Apicomplexa), a coccidian parasite in the swimbladder of cichlid fish. Z. Parasitenkd. **71,** 199–212.

Léger, L. and Bory, T. (1932): *Eimeria pigra* n. sp. C. R. Séances Acad. Sci. (Paris) **194,** 1710–1712.

Léger, L. and Hollande, G. C. (1922): Coccidie de l'intestin de l'anguille. C. R. Séances Acad. Sci. **175,** 999–1002.

Levine, N. D. (1983): The genera *Barrouxia*, *Defretinella*, and *Goussia* of the Coccidian family Barrouxiidae (Protozoa, Apicomplexa). J. Protozool. **30,** 542–547.

Levine, N. D. (1984): The genera *Cryptosporidium* and *Epieimeria* in the Coccidian family Cryptosporidiidae (Protozoa: Apicomplexa). Trans. Am. Microsc. Soc. **103**, 205–206.

Li, L. and Desser, S. S. (1985*a*): The protozoan parasites of fish from two lakes in Algonquin Park, Ontario. Can. J. Zool. **63**, 1846–1858.

Li, L. and Desser, S. S. (1985*b*): Three new species of *Octosporella* (Protozoa: Coccidia) from cyprinid fishes in Algonquin Park, Ontario. Can. J. Zool. **63**, 1859–1862.

Molnár, K. and Baska, F. (1986): Light and electron microscopic studies on *Epieimeria anguillae*, a coccidium parasitizing the European eel, *Anguilla anguilla*. J. Fish Dis. **9**, 99–110.

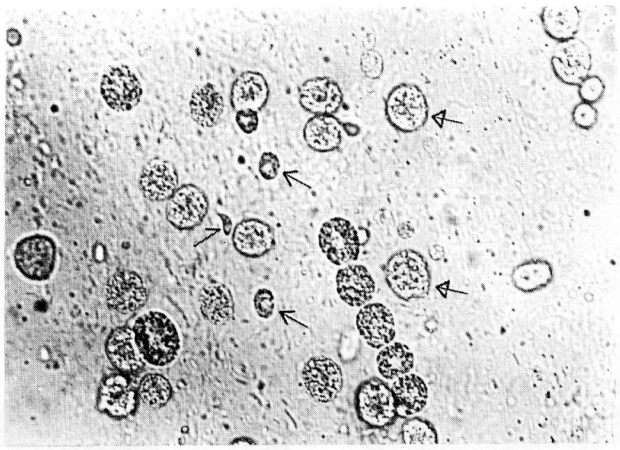


Fig. 1. Unsporulated oocysts in the intestine of sterlet. ↑: *G. vargai* oocysts; ↑: *G. acipenseris* oocysts; ↑: merozoite. Native preparation, × 700

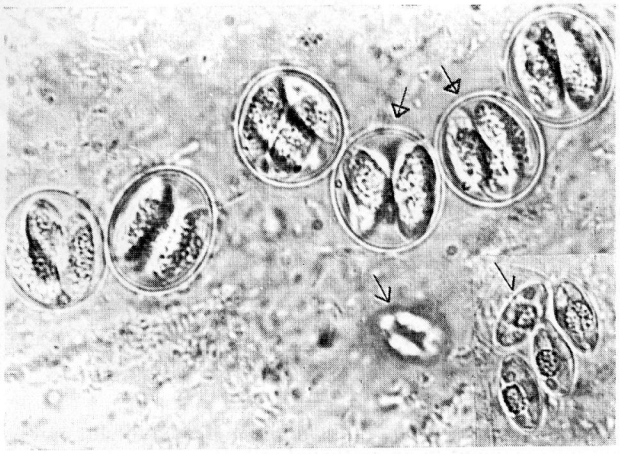


Fig. 3. Sporulated oocysts. ↑: *G. vargai* cysts after sporulation for 48 h; ↑: *G. vargai* sporocysts after sporulation for 72 h; ↑: *G. acipenseris* oocyst. Native preparation, × 1500

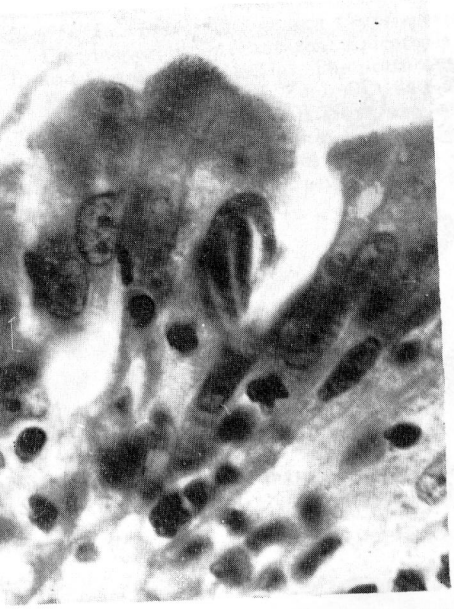


Fig. 4. G. vargai merozoites in an epithelial cell. Haematoxylin and eosin, × 1500

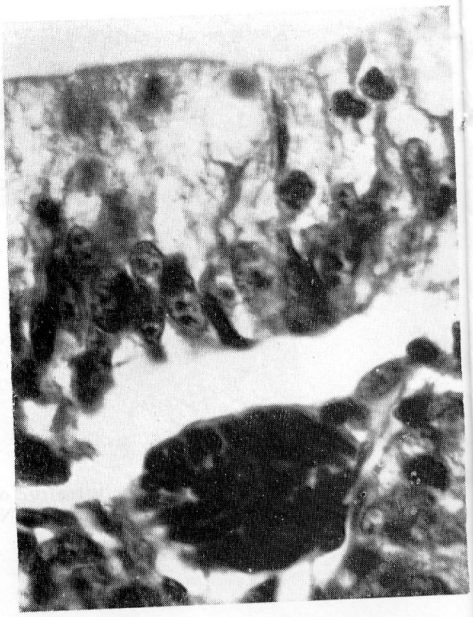
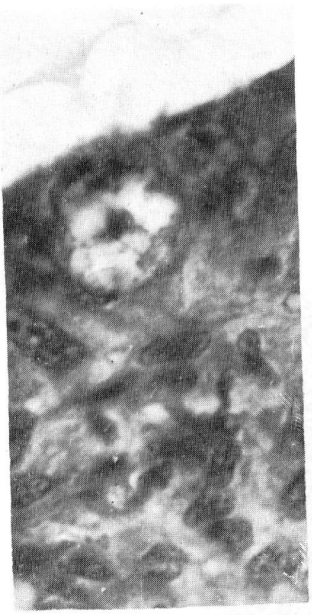
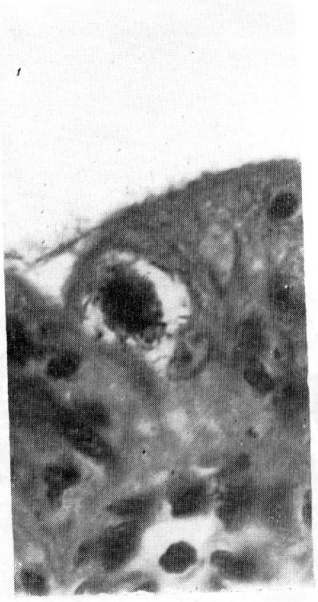


Fig. 5. G. vargai meront deep in the epithelium. Haematoxylin and eosin, × 1500





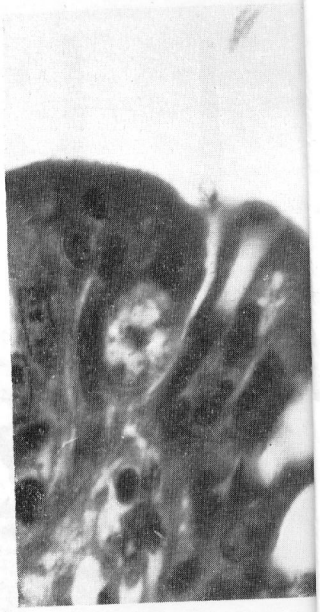


Fig. 6. G. vargai macrogamont in an epithelial cell. Haematoxylin and eosin, × 1500
Fig. 7. G. vargai microgamont in an epithelial cell. Haematoxylin and eosin, × 1500
Fig. 8. G. vargai macrogamont, situated basal to the nucleus of the epithelial cell. Haematoxylin and eosin, × 1500

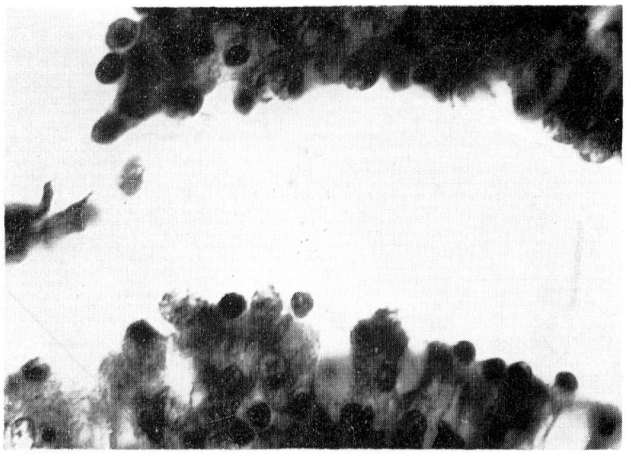


Fig. 10. G. acipenseris trophozoites in epiplasmal position. Haematoxylin and eosin, × 1500

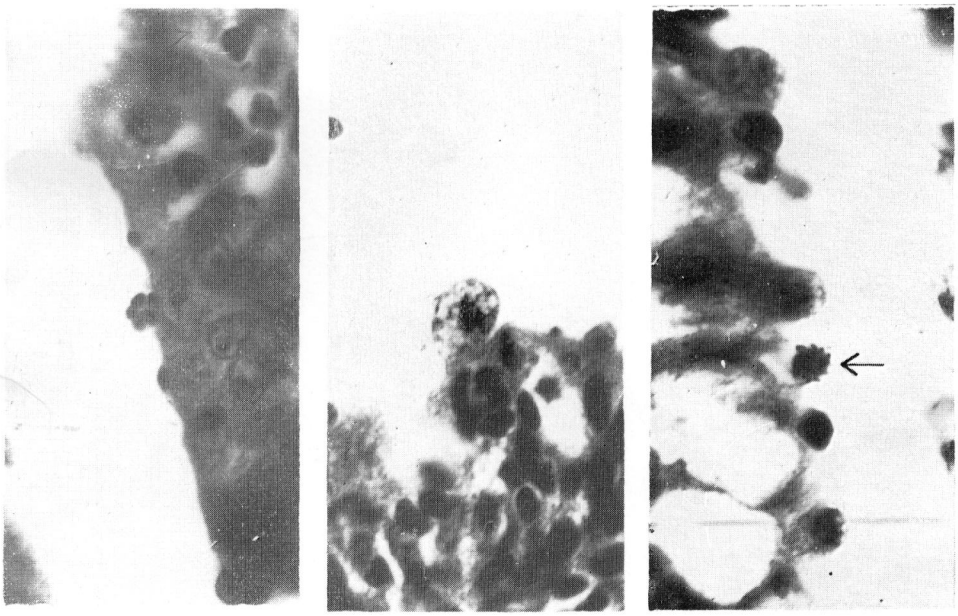


Fig. 11. G. acipenseris meront, apparently in extracellular position. Haematoxylin and eosin, × 1500

Fig. 12. G. acipenseris macrogamont. Haematoxylin and eosin, × 1500

Fig. 13. G. acipenseris microgamont (↑). Haematoxylin and eosin, × 1500