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The life cycle of *Eimeria danailovi* from ducks

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Abstract. The life cycle of *Eimeria danailovi* Gräfner, Graubmann et Betke in experimentally infected ducks was studied by optical microscopy. The asexual generation developed in the posterior part of jejunum and in the whole ileum. The sexual stages occurred in jejunum and ileum, and, in addition, in cecum and colon. All endogenous stages were localized in the cytoplasm of epithelial cells in the apical and basal parts of villi. Two generations of meronts were found to develop, differing from one another in the number of merozoites. The meronts of the first generation were observed 2 days post infection (DPI) and contained 10–14 (on the average 11) merozoites. The second generation of meronts, containing 12–22 (on the average 16) merozoites, developed on 3 DPI. The sexual stages were found in histological preparations on 5 and 6 DPI. They appeared in the faeces of experimentally infected ducks first on 5 DPI and they were shed for three days. Oocyst sporulation at room temperature lasted 2–3 days.

Gajadhar et al. (1983) recorded 22 coccidium species in ducks. Fujomoto et al. (1989) found another two *Eimeria* sp. in pintail (*Anas acuta* L.). The majority of these coccidia are known only on the basis of the description of their oocyst morphology. Detailed studies of their life cycles and pathogenicity for hosts have been made only in some species coming from domestic ducks. A high pathogenicity, especially for ducklings, was demonstrated in *Tyzzeria perniciosa* Allen (Allen 1936, Davies 1957, Versényi 1967). Bird deaths were caused also by *Eimeria danailovi* (Gräfner et al. 1965) and *Wenyonella philiplevinei* Leibovitz (Leibovitz 1968).

Gräfner et al. (1965) found *E. danailovi* oocysts in domestic ducks. They described the morphology and localization of endogenous stages found in dead or seriously ill birds after spontaneous infection. Oocysts of the same species were found also by Chauve et al. (1989) in France.

Since the present knowledge of *E. danailovi* life cycle is incomplete, the aim of this study was to provide some detailed information about this coccidium in an experimental infection.

MATERIALS AND METHODS

Ducklings at the age of three weeks were used for the experiments. After hatching they were kept in an isolated room and fed with VKCH-1 nonmedicated pellets. In order to exclude possible infection with coccidia, the duck faeces were floated by Sheather's sugar solution (500 g sugar, 6.5 g phenol, 320 ml distilled water) before the experiment.

E. danailovi oocysts were obtained from spontaneously infected ducks. After sporulation in 2% potassium dichromate and floatation in Sheather's solution the oocysts were kept in 2% potassium dichromate at 4 °C. Two weeks before the experiment they were multiplied by passage through 3-week-old ducklings.

Twelve of the 15 ducklings used were infected and three of them served as controls. The birds killed on 1 and 2 DPI were inoculated orally with the dose of 1.10^6 oocysts, those killed on 3 and 4 DPI with the dose of 5.10^5 oocysts, and those killed on 5 and 6 DPI with the dose of 25.10^4 oocysts. The ducks were killed at intervals of 12 h. Coprological examinations were made daily.

Nine samples were taken from the intestine (1 from duodenum, 2–5 from jejunum, 6–7 from cecum, and 9 from colon). The material for histological studies was fixed in 10% neutral formaldehyde and processed by a routine paraffin technique, sectioned 4–5 μm, and stained with Harris' haematoxylin and eosin. The material for semithin sections was fixed in 4% buffered paraformaldehyde (pH 7.2–7.4), postfixed in 2% OsO_4 in cacodylate buffer, dehydrated through acetone series and mounted into Durcupan ACM. The semithin sections were stained with toluidine blue and Warmke's polychrome (Warmke and Shen-Ling 1976). Mucosal smears were made from every part of the intestine and stained by Giemsa's method.

RESULTS

Oocyst morphology

The oocysts were ovoid, measuring 20.0 × 14.0 μm (16.8–22.9 × 12.0–16.0 μm), with a 1 μm thick wall and 3.0 μm (2.5–3.3 μm) wide micropyle covered by a slightly arched micropyle cap. One or two polar granules were situated near the micropyle. The residual body was absent. The sporocysts were ovoid, measured 10.8 × 6.3 μm (9.0–12.0 × 5.0–7.6 μm) and contained small Stieda bodies. The residual sporocyst body was spherical and compact and was localized centrally (Figs. 1, 2).

Localization of endogenous stages

The development of asexual generation took place in the posterior part of jejunum and along the whole length of ileum. The sexual stages occurred in jejunum and ileum and, in addition, in cecum and colon. All asexual stages were localized in the cytoplasm of epithelial cells in both apical and basal parts of villi.

Life cycle

The measurements of all endogenous stages are given in Table 1. The development of meronts in the cytoplasm of epithelial cells of intestine was observed on 2 and 3 DPI, sporadically also on 4 DPI. The meronts and merozoites found on 2 and 3 DPI differed in their size (Table 1). However, the main difference between the meronts consisted in the number of merozoites contained in them (Table 1, Figs. 3, 4). The meronts found on 2 DPI contained 11 merozoites, while those found on 3 DPI contained 16 merozoites on the average. The first stages of sexual multiplication were found on 4 DPI and their development lasted till 5 DPI. Larger microgametocytes were oval, like the macrogametocytes.

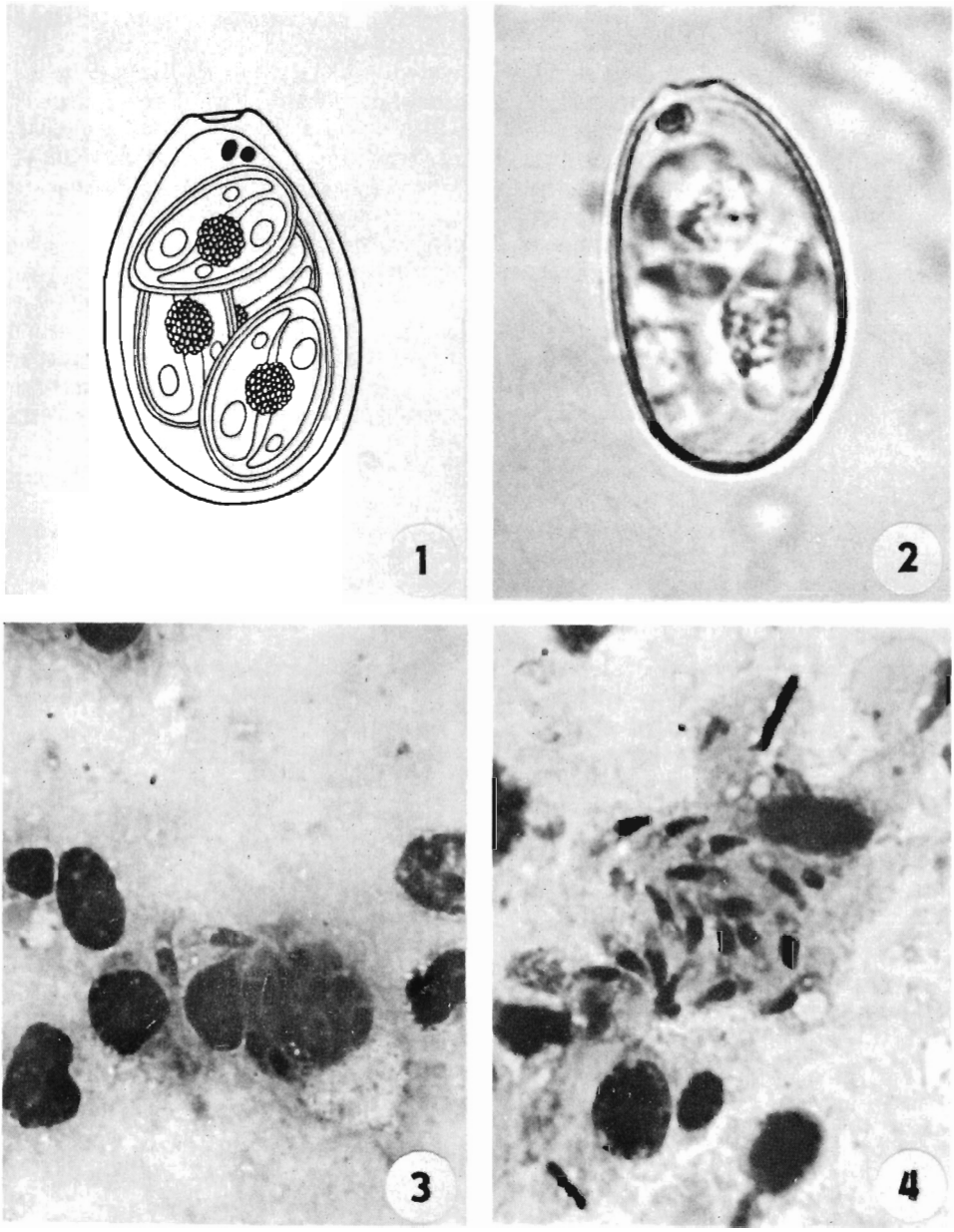
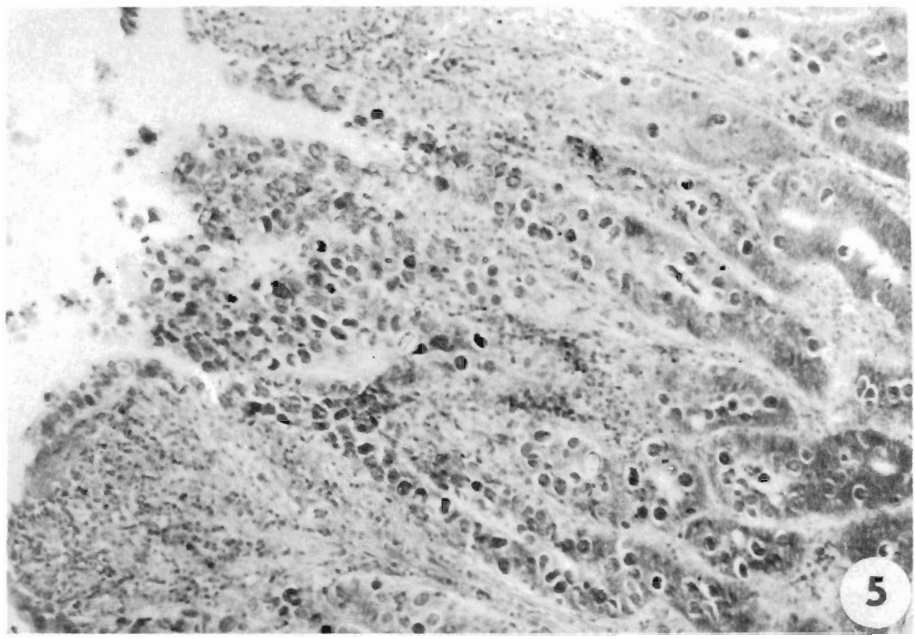
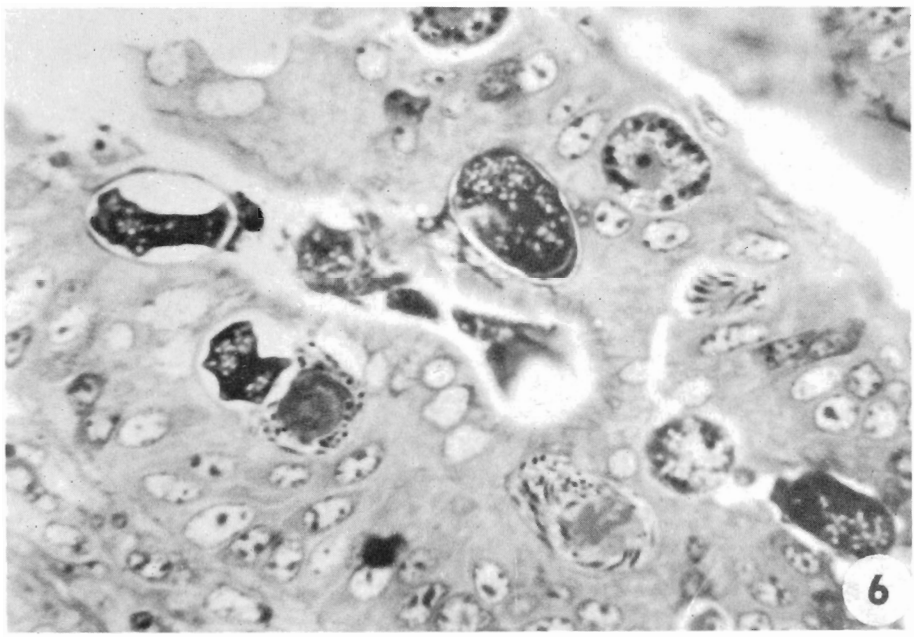


Fig. 1. *E. danailovi* oocyst (reconstruction). **Fig. 2.** *E. danailovi* oocyst (native, × 2600). **Fig. 3.** First generation of merozoites on 2 DPI (Giemsa, × 1400). **Fig. 4.** Second generation of merozoites on 3 DPI (Giemsa, × 1400).





Figs. 5, 6. Sexual developmental stages and oocysts in the cytoplasm of epithelial cells of small intestine on 5 DPI. **Fig. 5** – Harris' hematoxylin-eosin (× 125); **Fig. 6** – semithin section, polychrome staining after Warmke (× 900).

Table 1. Measurements of endogenous stages of *E. danailovi*

Developmental stage	Size (μm)	Mean size (μm)	No. of merozoites in a meront
Meront on 2 DPI	8.9–14.4 × 6.0–9.2	11.1 × 8.3	
Meront on 3 DPI	9.1–15.8 × 7.2–14.0	12.3 × 10.1	
Merozoite on 2 DPI	6.1–9.5 × 1.6–2.4	7.2 × 2.0	10–14, mean 11
Merozoite on 3 DPI	6.0–7.6 × 1.6–2.3	6.5 × 1.9	12–22, mean 16
Macrogametocyte	10.2–16.8 × 9.1–13.3	13.6 × 11.2	
Microgametocyte	12.2–19.9 × 9.0–13.0	15.7 × 10.2	

The oocysts were found in histological and semithin sections 5 and 6 DPI (Figs. 5, 6). In the faeces of experimentally infected ducks, they appeared for the first time on 5 DPI. Their maximum shedding occurred on this and the following day. On 7 DPI, the number of oocysts shed decreased. No oocysts were found either in the intestinal contents or intestinal mucosa smears of a duck killed on 8 DPI. The oocyst sporulation was completed at room temperature after 3–4 days.

DISCUSSION

The endogenous developmental stages of *E. danailovi* have been studied only by Gräfner et al. (1965). The authors examined spontaneously infected birds without giving any detailed information about the time course of the cycle and prepatent and patent periods of this coccidium.

The results of our experiments show that two meront generations develop in *E. danailovi* differing from one another particularly in the number of their merozoites. In the first generation, there were 10–14 merozoites per meront and in the second, 12–22 merozoites per meront. Since Gräfner et al. (1965) described only one type of meronts with 8 merozoites, it is probable that the meronts of the first generation were involved in this case. The merozoites found by them measured 6.24–8.32 × 1.56–2.08 μm, which corresponds to the measurements of the merozoites in our material (Table 1). Table 1 also shows slight differences in the measurements of sexual stages compared to the results obtained by Gräfner et al. (1965) who described microgametocytes measuring 10.48 × 12.48 μm and macrogametocytes measuring 6.24 × 8.32 μm.

As it follows from our experiments, the development of the asexual generation takes place mainly in the regions of jejunum and ileum. The sexual stages can be found also in cecum and colon. Gräfner et al. (1965) found sexual stages in addition to jejunum and ileum also in duodenum, which might have been caused by the fact that they examined only very weakened birds exhibiting clinical symptoms of the disease.

The first oocysts were found in the faeces of experimentally infected ducks on 5 DPI. They were shed for three days. The complete oocyst sporulation occurred

on 3–4 DPI, which was in agreement with the results obtained by Gräfner et al. (1965), who recorded a 4 days' sporulation.

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