Life cycle and ultrastructure of *Eimeria stigmosa*, the intranuclear coccidian of the goose (*Anser anser domesticus*)

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Abstract. The oocyst morphology and endogenous development of *Eimeria stigmosa* Klimeš, 1963 in a domestic goose (*Anser anser domesticus* L.) was studied using light and electron microscopy. The oocyst wall consisted of two layers. The outer layer formed densely accumulated protrusions, whilst the inner layer was smooth and formed a collar structure around the micropyle. Meronts were observed in the posterior part of the jejunum, along the whole length of the ileum and in Meckel's diverticulum within 1–4 days post infection (DPI). Sexual generation was found in the caecum and colon 4–5 DPI. All endogenous stages were located intranuclearly in enterocytes, predominantly in the apical part of the villi in distinct parasitophorous vacuoles. Numerous different developmental stages were frequently found in one nucleus. Current evidence indicates the existence of only one asexual generation formed by ectomerogony. During the development of asexual stages, invaginations into the body of a meront by the host cell nucleoplasm were observed.

The species *Eimeria stigmosa* was first identified in the domestic goose (*Anser anser domesticus L.*) by Klimeš (1963) in Czechoslovakia. He observed sexual stages in the epithelial cells of the intestinal villi. Since then several authors have acknowledged the occurrence of this parasite in geese (Friedhoff et al. 1983, Xie et al. 1986, Chauve 1988). The life cycle of *E. stigmosa* has been studied by Gajadhar et al. (1986) who demonstrated that the development of endogenous stages took place within nuclei of epithelial cells.

In the present paper, oocyst morphology and ultrastructure of endogenous stages of *E. stigmosa* are described.

MATERIALS AND METHODS

Sixteen 3-week-old goslings were kept in an isolated room after hatching and fed medicated pellets VH1. Two days before the inoculation and during experiments, the goslings were provided with a wheat pollard.

The oocysts of *Eimeria stigmosa* were obtained from the faeces of naturally infected geese and repeatedly given to experimentally infected 3-week-old goslings. After sporulation in 2% potassium dichromate, the oocysts were concentrated in Sheather's sugar solution (500 g sugar, 6.5 g phenol, 320 ml distilled water) by flotation, and stored in 2% potassium dichromate at 4 °C for 2–4 weeks.

Twelve of the 14 goslings used were infected and two of them served as controls. The birds killed 1–2 DPI were inoculated orally with 1.10^6 oocysts, those killed 3–4 DPI with 5.10^5 oocysts, and those killed 5–6 DPI with 25.10^4 oocysts. The goslings were killed at intervals of 12 hours. They were coprologically examined every day.

Samples from the duodenum, jejunum, ileum, caecum and large intestine were fixed with 4% glutaraldehyde in cacodylate buffer (pH 7.2), and then postfixed with 2% osmium tetroxide in the same buffer, dehydrated in acetone and embedded in Durcupan ACM. The semithin sections were stained with toluidine blue and Warmke's polychrome (Warmke and Lee 1976). Ultrathin sections were contrasted using an uranyl acetate and lead citrate, and examined with a Philips EM 420 electron microscope. Mucosal scrapings were taken from every part of the intestine and stained after Giemsa.

For scanning electron microscopy (SEM), the oocysts were fixed in 10% neutral formaldehyde, dehydrated in a graded series of ethanol, dried using CO_2 critical point, coated with gold and examined with TESLA BS 300 scanning electron microscope. Sodium hypochlorite solution (5% w/v) was used to break the outer layer of the oocyst wall.

RESULTS

I. Oocyst morphology

Oocysts of *E. stigmosa* (Fig. 1) were oval to slightly ovoid, measuring 23.2×17.4 $(20.7 - 25.0 \times 15.0 - 20.0)$ μm, and were without an oocyst residuum. One or two polar granules were situated near the 2.6 (2.0–3.3) μm wide micropyle. The 1.6 (1.2–2.2) μm thick oocyst wall was composed of two layers; an inner layer, thickened at the micropyle and an coarse outer layer. By means of SEM we observed that the external surface of the oocyst wall was dotted with a great many protuberances (Fig. 2). The inner smooth layer formed a clearly visible collar structure around the micropyle (Fig. 3). The sporocysts measuring 11.7×8.0 $(10.0 - 12.6 \times 6.9 - 9.2)$ μm were ovoid with a small Stieda body at the narrow end and contained a diffuse granular residuum.

II. Endogenous stages

Localisation

The development of all endogenous stages took place in the posterior part of jejunum, along the whole length of ileum and in Meckel's diverticulum. Sexual stages also occurred in the caecum and colon. Both asexual and sexual stages were located within the nuclei of enterocytes, predominantly in the apical part of the villi.

Light microscopy

The measurements of all stages of the endogenous cycle are given in Table 1.

First developing meronts were sporadically observed 1 DPI. They were round in shape, had a small nucleus and were enclosed within a parasitophorous vacuole. As many as 8 meronts could be found in one nucleus. During the growth of these stages, a hypertrophy of the nucleus of the host cell occurred (Fig. 13). From 1.5 to 4 DPI peaking occurrence at 2 and 3 DPI mature, oval meronts were observed.

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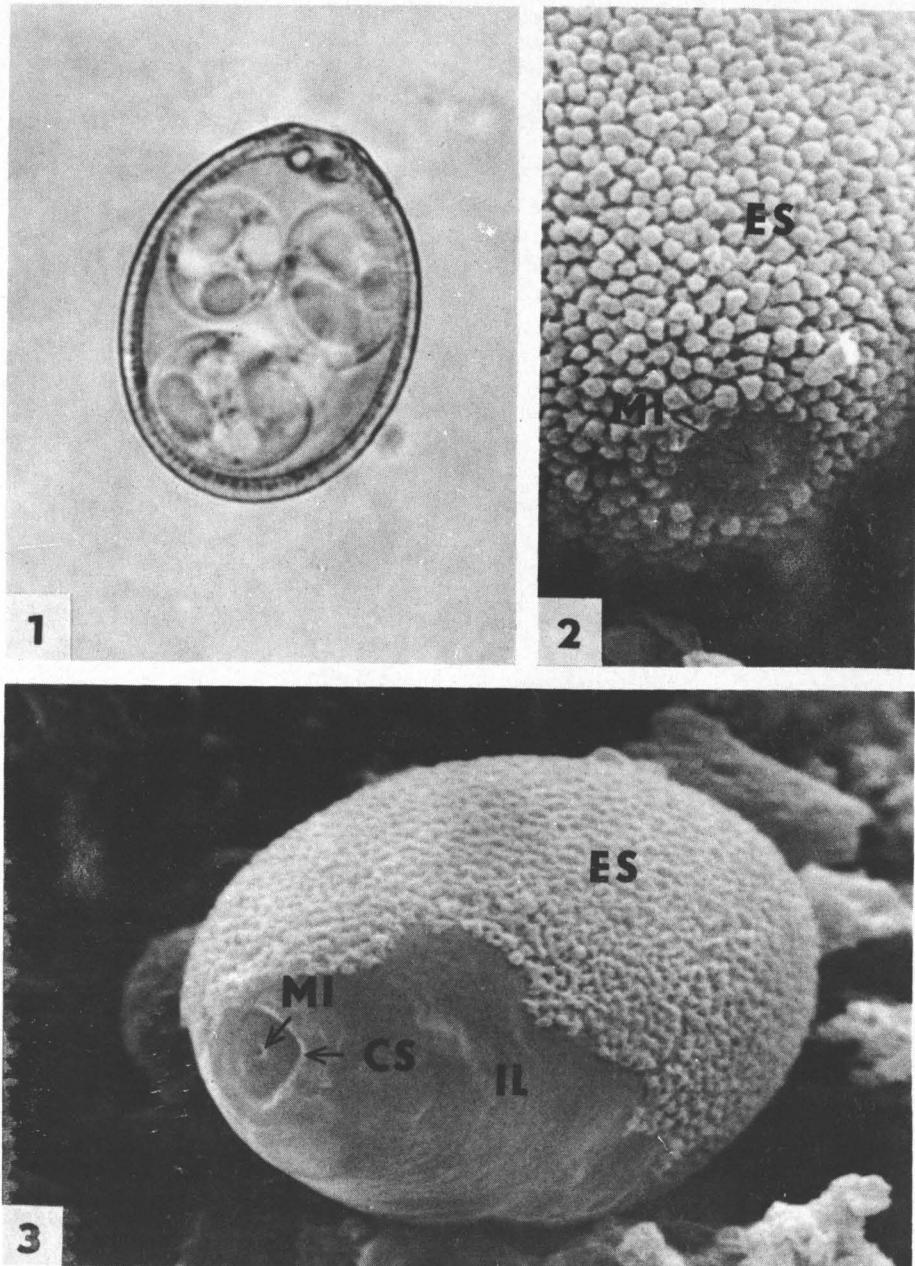
Fig. 1. Sporulated oocyst of *E. stigmosa* (native, ×2,200).
Figs. 2, 3. Scanning electron micrographs of an oocyst of *E. stigmosa*. Note the bumpy external surface (ES), the smooth inner layer (IL), and the collar structure (CS) around the micropyle (MI) (Fig. 2. ×6,500; Fig. 3. ×4,000).
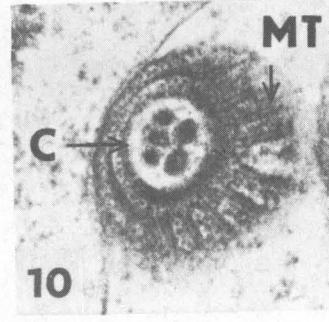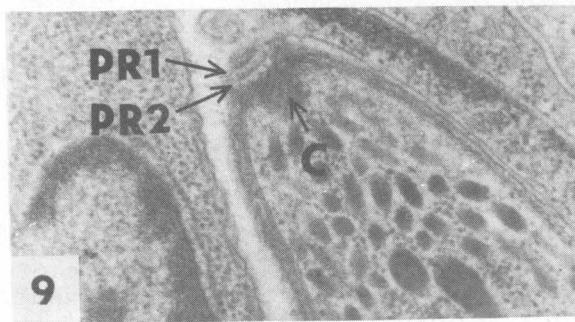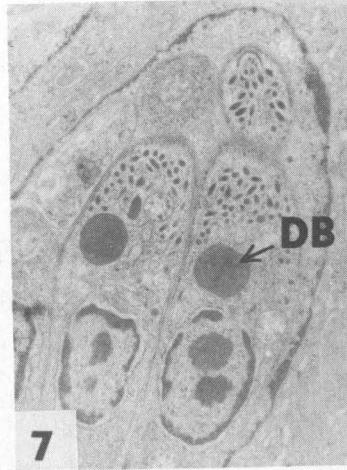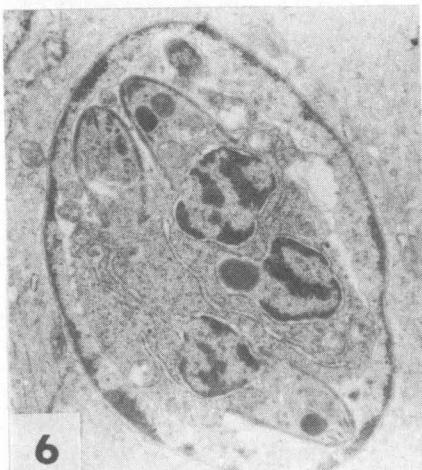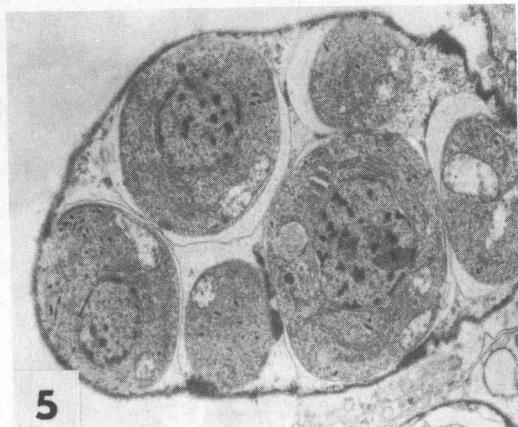
Figs. 4–10. Ultrathin sections of endogenous stages of *E. stigmosa* in the nucleus of the host cell. **Fig. 4.** Sporozoite finishing penetration into the host cell nucleus ($\times 12{,}300$). **Fig. 5.** Six immature meronts in one nucleus; every meront has its own parasitophorous vacuole ($\times 8{,}200$). **Fig. 6.** Merozoite formation by ectomerogony ($\times 10{,}500$). **Fig. 7.** Merozoites with electron dense bodies (DB) ($\times 10{,}500$). **Fig. 8.** Section through a micropore (MP) of a merozoite ($\times 45{,}000$). **Fig. 9.** Section through the apical pole of a merozoite (PR1, PR2 – preconoidal rings, C – conoid) ($\times 36{,}000$). **Fig. 10.** Cross section through the anterior end of a merozoite (C – conoid, MT – microtubules) ($\times 72{,}000$).
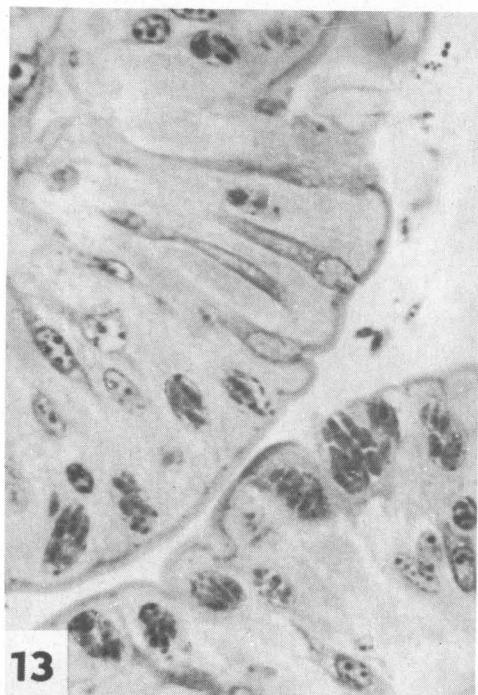
Figs. 11–14. Endogenous stages of *E. stigmosa* in the nucleus of the host cell.
Fig. 11. Mature meront with twelve merozoites (TEM, ×6,350). **Fig. 12.** Two meronts of a different developmental level in one nucleus (TEM, ×9,700). **Fig. 13–14.** Migration of infected nuclei from the basal lamina to the laminal striated border and the release of merozoites to the intestinal lumen (Fig. 13 – semithin section stained with Warmke's polychrome, ×1,800; Fig. 14 – TEM, ×13,500).

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However, they did not differ morphologically and contained the average number of uninucleate merozoites which are usually found in these meronts.

Sexual developmental stages were found 4–5.5 DPI. Mature macrogamonts were oval and smaller than microgamonts. The micro- and macrogamonts occasionally developed simultaneously in one nucleus.

Table 1. Endogenous stages of *Eimeria stigmosa*

Stage	Size (μm)	Mean size (μm)	No. of merozoites in one meront (mean)
Meront 2 DPI	7.5–9.8 × 4.0–5.6	9.2 × 5.2	11–19 (12)
Meront 3 DPI	8.0–11.5 × 4.2–7.0	9.5 × 5.5	9–18 (12)
Merozoite 2 DPI	5.5–8.0 × 1.1–2.2	6.4 × 1.6	
Merozoite 3 DPI	5.2–6.6 × 1.2–2.3	6.1 × 1.6	
Macrogamont	14.1–16.3 × 9.0–12.7	15.9 × 10.7	
Microgamont	18.0–25.4 × 10.0–18.0	21.2 × 13.65	

In histological sections, oocysts were observed at 5 and 6 DPI, which was the last when gosling was killed. In the faeces of experimentally infected goslings, they appeared at 5 DPI and continued to be shed for 4 days. At room temperature, the oocyst sporulation was completed after 2–3 days.

Transmission electron microscopy

The first sporozoites completing the penetration process into the nucleus were observed sporadically 1 DPI. Components of the apical complex were still retained in the early course of infection after sporozoite penetration into the host nucleus (Fig. 4). During the subsequent development, sporozoites became more round and the apical complex disappeared. Using both the electron and light microscopes, we observed several different stages in one nucleus. Each meront had its own parasitophorous vacuole and between the meronts, a thin layer of the host cell nucleoplasm was evident (Fig. 5).

Merozoites were formed by ectomerogony (Fig. 6) and their ultrastructure was typical of coccidial merozoites. They had a tri-membraneous pellicle, a posterior nucleus with a prominent nucleolus, a Golgi apparatus, micropores, and endoplasmic reticulum. Their apical complex was formed by the usual organelles. In addition, there was one large, oval-shaped body above the nucleus (Fig. 7–11). Meronts at different phases of development were frequently found in one nucleus. After the formation of merozoites these meronts were separated by a single plasma membrane (Fig. 12). During this formation of merozoites in a meronts, the infected nucleus enlarged, and it was shifted towards the periphery of the host cell and the nuclear and cytoplasmic membranes of the cell ruptured. Merozoites were released and entered the intestinal lumen (Figs. 13, 14).
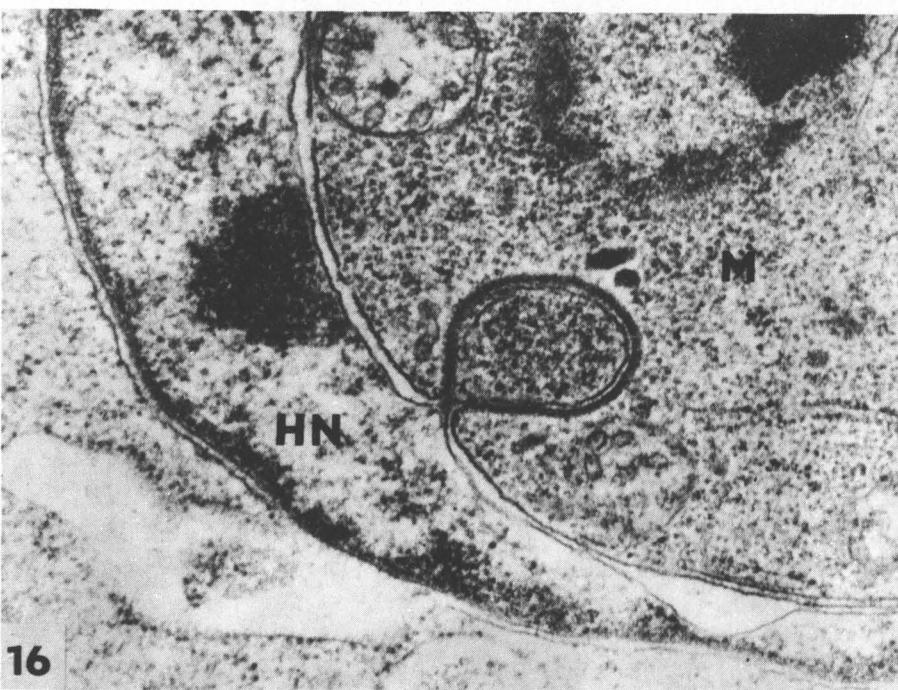
Fig. 15. Immature gamont in the nucleus of the host cell (TEM, ×15,400). **Fig. 16.** Suction of host nucleoplasm (HN) into the cell of meront (TEM, ×31,700).

Gametocytes also developed within a parasitophorous vacuole limited by a unit membrane (Fig. 15). During the development of asexual stages, invaginations into the body of a meront by the host cell nucleoplasm were noticed (Fig. 16).

DISCUSSION

The structure and size of oocysts studied in the present paper corresponded with the description of two species – *E. stigmosa* Klimeš and *E. striata* Farr (Farr 1953, Klimeš 1963). Oocysts of the two species differ mainly in the character of the oocyst wall. The wall of *E. striata* oocyst is pitted and finely striated whilst the surface of *E. stigmosa* is covered with numerous "dark" as well as "light" punctations (as seen via the light microscope). On the basis of the oocyst surface structure, we identified the oocysts found by us as *E. stigmosa*. They differed somewhat in their distinct bumpy surface and in the formation of the area around the micropyle from the description given by Gajadhar et al. (1986), who also examined oocysts of *E. stigmosa* by means of SEM and noted differences in the thickness of the oocyst wall as well. Klimeš (1963) found the oocyst wall to be 1.2–2.0 μm thick – which tallies with our results (1.2–2.2 μm) – whereas Gajadhar et al. (1986) reported a 1 μm thick oocyst wall.

In birds, the intranuclear development was observed only in the coccidia of geese. It is interesting that five out of nine species with an endogenous cycle have so far been shown to develop intranuclearly. The schizogony of *E. hermani* Farr and *E. nocens* Kotlán, gametogony of *Tyzzeria parvula* Klimeš and both schizogony and gametogony of *E. kotlani* Gräfner et Graubmann and *E. stigmosa* occur within the nuclei of gut epithelial cells in geese (Antukhaev 1976, 1977, Ponizovskii and Shibalova 1978, Shibalova and Morozova 1979, Gajadhar et al. 1986, Skene and Fernando 1990).

The life cycle of *E. stigmosa* is described in two reports: Klimeš (1963) only found sexual stages, Gajadhar et al. (1986) observed both asexual and sexual part of the endogenous development. Confirming the results of Gajadhar et al. (1986) we observed meronts 1.5–4 DPI which supposes the existence of a single asexual generation. However, in our experiments, the prepatent period was 5 days with excretion of oocysts continuing for 4 days. This corresponds with the results given by Klimeš (1963) who observed the first oocysts appearing in faeces 5 DPI and their discharge lasting for 2–3 days. On the other hand, Gajadhar et al. (1986) noted that the average prepatent period was 6 days and all oocysts were produced within approximately 24 hours.

By means of transmission electron microscopy (TEM), we found that all endogenous stages of *E. stigmosa* are located within a distinctly developed parasitophorous vacuole. The presence of a parasitophorous vacuole surrounded by a unit membrane was revealed in most intranuclear coccidia investigated by TEM (Shibalova and Morozova 1979, Entzeroth and Scholtyseck 1984, Daou-

di 1987, Skene and Fernando 1990). By contrast, Gajadhar et al. (1986) reported that the meronts and gamonts from *E. stigmosa* lacked a parasitophorous vacuole and were in close apposition with the host nucleoplasm.

On the basis that a parasitophorous vacuole was absent during the endogenous development and that there were certain differences in oocyst morphology between the coccidia studied by Gajadhar et al. (1986) and the ones dealt with in the present paper, we cannot exclude the possibility that two different species of the genus *Eimeria* were studied.

Typical of the merozoites from *E. stigmosa* is the presence of an oval-shaped, electron dense body situated in the anterior part of the cell. Similar electron dense bodies, somewhat resembling the refractile bodies of sporozoites, have been also reported in merozoites of *E. mitis* Tyzzer and *E. mivati* Edgar et Seibold from chickens (Fitz-Coy et al. 1989) and from type-A merozoites of *Globidium*-cysts from goats (Mehlhorn et al. 1984).

We frequently found numerous meronts and gamonts in one nucleus which were in different stage of development. In the coccidia during the intranuclear development however this is apparently a usual phenomenon. Similar observations were also described by Ponizovskii and Shibalova (1978) in *E. nocens*, Shibalova and Morozova (1979) in macrogametes of *T. parvula*, and Davis et al. (1957) in *E. alabamensis* Christensen.

During the development of the endogenous stages in *E. stigmosa*, infected nuclei were shifted towards the periphery of the host cells. A similar phenomenon has been reported in *Isospora mesnili* Sergent, *I. manchacensis* Atkinson et Ayala and *E. hermani* (Sergent 1902, Atkinson and Ayala 1987, Skene and Fernando 1990).

The problem with intranuclear feeding of coccidian parasites has not been resolved until now. Shibalova and Morozova (1979), who studied the development of *T. parvula*, observed deep invaginations on the surface of macrogametes, in which fragments of host cell nucleoplasm were invaginated. They thought that this may be connected with the process of parasite feeding habits. The results of our electron microscopic examination of the asexual stages of *E. stigmosa* revealed a similar engulfment of host nucleoplasm into the cell of the parasite. As the nucleoplasm is reduced during the development of parasites, we agree with Shibalova and Morozova's opinion that coccidia with an intranuclear development utilize the host cell nucleoplasm for their nutrition.

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