LIFE CYCLE OF EIMERIA COECICOLA CHEISSIN, 1947

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Abstract. Life cycle of Eimeria coecicola was studied in experimentally infected rabbits by light microscopy and by transmission and scanning electron microscopy. First and second generation meronts developed in the vermiciform appendix; third and fourth generation meronts were located in the epithelium of the ileum. Gametogony developed again in the vermiciform appendix. The prepatent period was 9 days. New data were obtained by the study of asexual reproduction. First generation meronts were first observed 4 days post infection (DPI), which is relatively late in comparison with other species of rabbit coccidia. Sporozoites were found in lymphatic follicles of the vermiciform appendix at 4 DPI by transmission electron microscopy. This suggests, together with selective location of first generation meronts in the epithelium adjacent to these follicles, that major part of sporozoites enter the epithelial cells through lymphatic follicles and not through the lumen of the vermiciform appendix. The process of development of first generation merozoites is similar to endodyogeny. The differences are in formation of apical parts of daughter merozoites which is not coincidental with nuclear division and in formation of the outer membrane of pellicle which arises within the mother cell. Some first generation merozoites have 2–3 nuclei, second and fourth generation merozoites are only multinucleate, while third generation merozoites are only multinucleate. We found that further merozoites are formed in multinucleate third generation merozoites by endodyogeny.

The life cycles of rabbit coccidia were described almost in all valid species, with the exception of Eimeria coecicola, the biology of which was described only by Cheissin (1947, 1967, 1968). Cheissin (1947, 1967) described meronts found in the ileum within 18 cm from ileoceleal valve. Meronts observed at 6 DPI measured 12–18 μm and had 8–12 merozoites. Gamonts developed in the vermiform appendix and in the caecum from 7 DPI; first oocysts appeared in the faeces at 9 DPI. In addition to these stages Cheissin (1968) described also meronts found in the vermiform appendix visible until 9 DPI. Meronts measured 12–15 μm and produced elongate merozoites (10×0.8 μm). The whole endogenous cycle of E. coecicola was described insufficiently; neither the number of asexual generations, nor morphology of the endogenous stages of E. coecicola is known.

MATERIALS AND METHODS

Oocysts were put to sporulate in a 2–3 mm deep layer of 2.5% potassium dichromate in Petri dishes at room temperature.

Material used for inoculation was obtained from spontaneously infected rabbits (Oryctolagus cuniculus). Suspension of sporulated oocysts was diluted appropriately and then dropped on the slides. Individual drops were examined microscopically. Each drop containing only oocysts of E. coecicola was collected using a thin capillary tube. A total of 100 oocysts was collected and the suspension was diluted with five drops of water. Then a 5-week-old, coecisida-free rabbit was orally inoculated.

Oocysts obtained from the faeces of this rabbit were used to study endogenous cycle.

Oocysts submitted for inoculation were cleaned and concentrated. An oocysts suspension was centrifuged, oocysts were once washed. The sediment was stirred in a 3M solution of saccharose and centrifuged at 750 g. The supernatant was diluted at least with a fivefold water volume and centrifuged. In this manner oocysts remained in the sediment. The size of inoculum was counted using a counting chamber working on a Mac Master chamber principle. Rabbits were inoculated with corresponding oocysts doses into the stomach via a catheter.

Crossbreds of Chinchilla and Californian White rabbits were used in the experiments. Females were housed with their young weaned rabbits in wooden cages, regularly cleaned with boiling water. The animals were fed pellets KO 16 and received only boiled water. They were treated with Sulfa-
hombin at a dose of 25 ml of water, which was discontinued 3 days before inoculation. Rabbits aged 3 weeks were used in experiments and were housed individually in metal cages cleaned daily with boiling water including water bottles and food dishes. The animals were coprocystically examined each day during the whole experiment.

Rabbits were inoculated with oocysts of *E. cuniculi*; the doses were as follows: 10⁶ oocysts for rabbits killed at 3 and 5 DPI, 3 x 10⁶ oocysts for rabbits killed at 4 and 6 DPI, 3 x 10⁶ oocysts for rabbits killed at 5 and 7 DPI, 3 x 10⁶ oocysts for a rabbit killed at 6 DPI, and 3 x 10⁶ oocysts for a rabbit killed at 10 DPI. The whole experiment was repeated once more in the same arrangement. All rabbits were killed at 7 days after beginning of the experiment served as unoinoculated control during the whole experiment and the repeated one.

The animals were killed by ether. Samples from the small intestine were taken immediately behind the stomach and then at 10 cm intervals along its whole length (14–19 samples). The last sample was taken at the distance of 2–3 cm from the ileocecal valve. Four samples were taken from the cecum (1 sample from the region of appendix, then from the anterior, middle, and posterior part of cecum), three samples from the mesenteric appendix were taken from the anterior, middle, and terminal parts. One sample was taken from the cranial part of the colon and then at 10 cm intervals (5–8 samples). In addition, one sample from the jejunum and one sample from the veriform appendix were taken from each animal for examination by scanning electron microscopy (SEM).

One rabbit inoculated with 3 x 10⁶ oocysts and killed at 4 DPI, two rabbits inoculated with 3 x 10⁶ and 10⁶ oocysts and killed at 5 DPI, and finally two rabbits inoculated with 3 x 10⁶ oocysts and killed at 6 DPI were used for ultrastructural examination of developmental stages of *E. cuniculi*.

The material was fixed in 1% formaldehyde, processed by conventional paraffin technique and tissue sections, cut at 4 μm were stained with Harris’ haematoxylin-eosin. The material used for SEM was fixed in 4% buffered paraformaldehyde (pH 7.2–7.4), postfixed in 2% OsO₄, in cacodylate buffer and dehydrated in a graded series of ethanol. Then the material was embedded with 1:1 acetone—ethanol mixture and acetone, dehydrated using CO₂ critical point, mounted on stubs and coated with gold. A Tesla BS-300 scanning electron microscope was used for examination.

For transmission electron microscopy (TEM), the material was fixed and dehydrated in the same manner as for SEM, saturated with propylene oxide, a propylene oxide—Polybed 812 mixture and embedded in Polybed 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Philips EM 402 electron microscope.

**RESULTS**

**a) Light and scanning electron microscopy**

Oocysts which were under study (Pl. I, Fig. 1) correspond to that described by Cheisain (1947, 1967, 1968). They are oval to cylindrical, possessing a light yellow or light brown wall which is thickened around a micropyle. The sporocysts are oval, with a small Stieda body. The residual body of the oocyst is spherical, while sporocyst's residual body is of irregular shape. A total of 100 oocysts measured as follows:

**Oocysts:**

- 33–38 x 18–22 μm, with a mean value of 35.2 x 20.2 μm
- Residual body of the oocysts: 5–8 μm, with a mean value of 6.5 μm

**The width of the micropyle:** 2.5–5 μm, with a mean value of 3.1 μm

The measurements of all stages of the endogenous cycle are mentioned in Table 1. The endogenous stages were first observed at 4 DPI in the veriform appendix. The vermiform appendix in rabbits is the organ with a special histological structure (Snipe, 1978). Lymphatic follicles are therein present and they are located regularly, forming an approximately square net. The follicles are covered with a monolayer epithelium. Semi-rounded formations, domes are formed in these areas...
(Pl. I, Fig. 2). In other superficial parts mucosa overlaps the domes. From the viewpoint of the life cycle of *E. coeca* it is important to differentiate the mucous epithelium, which either possesses or not the lymphoepithelial tissue immediately below it. First asexual generation of this coccidian is selectively located in the epithelium of domes below which the lymphoepithelial tissue is immediately located. First-generation meronts occur first at 4 DPI, and they divide into two relatively thick merocysts (Pl. I, Fig. 2). The same meronts are found in the same location at 5 DPI, with the difference that some merocysts appear to be binucleate and trinucleate in comparison with the situation observed at 4 DPI (Pl. III, Fig. 1). The number of meronts with multinucleate merocysts can reach even 30% at 5 DPI as well as at 6 DPI. The number of binucleate merocysts was multiple larger than of trinucleate one. From 4 to 6 DPI the mean size of first generation merocysts increases (see Table 1).

From 4 to 7 DPI, with maximum occurrence at 6 DPI, second generation meronts containing long, slender merocysts were observed (Pl. III, Fig. 1). These are usually situated in a parallel arrangement and merocysts possess sporadically a small residual body. Second generation meronts are located similarly as first generation meronts in the epithelium of domes, and in the immediate vicinity of domes, too. Fourth and second generation merocysts were found in slits between domes and mucosa of the veriform appendix by means of SEM (Pl. IV, Fig. 3).

The developmental stages in the ileum were first observed at 6 DPI. We found third generation meronts located in the epithelium on the walls and tops of the villi, less frequently in the epithelium of the crypts. They contain merocysts mostly with 4 to 8 nuclei situated in a parallel arrangement or irregularly (Pl. V, Fig. 1). This type of meronts was observed predominantly at 6 and 7 DPI, less frequently at 8 DPI.

Fourth generation meronts were observed at 7 DPI and then always until 10 DPI with maximum occurrence at 8 DPI. These meronts contained large numbers of uninucleate merocysts, arranged usually in rosette-like patterns, which are compactly clustered (Pl. V, Fig. 4). Their nuclei are situated usually near the center of the meront. Second generation meronts first, a fourth asexual generation, especially in the size of meronts and number of merocysts in individual meronts. It depends on the intensity of infection in a given place. During heavy infection the size of meronts is smaller and number of merocysts in meronts in lower.

The number of asexual stages in the veriform appendix is lower at 7 DPI; young gamonts, located in the epithelium start to appear. They are found in the whole mucosal epithelium, infrequently in the epithelium of domes as in the first asexual generation. Asexual stages were observed scarcely in the veriform appendix at 8 DPI, only mature gamonts and first oocysts were found (Pl. VI, Fig. 1). Meronts of all generations can be located above or below the host cell nucleus, while gamonts are usually below it. By means of SEM we observed that the cytoplasmic membrane of the host cell together with an adjacent cytoplasmic layer got vaulted above the releasing oocyst. Microvilli disappeared, cytoplasmic membrane was expanded and finally broken (Pl. VI, Fig. 2).

b) Transmission electron microscopy

A part of endogenous cycle of *E. coeca* — sporozoites and first, second and third asexual generations were examined with the TEM. Sporozoites appeared at 4 DPI in the lymphoepithelial tissue, not in the epithelium, where the first generation meronts undergo their development. Sporozoites do not differ in their ultrastructure from commonly known coccidial sporozoites. A large refractile vacuole and amylopectin serving as a sparse substance were observed. Also some components of apical complex — micronemes and rhoptries were visible on well-oriented sections (Pl. I, Fig. 3). Sporozoites were always found within the host cells, adjacent to lymphocytes, which were surrounded by a close space formed by the parasitophorous vacuole.

Process of inner formation of two daughter merocysts was observed in first generation meronts. This process is quite different from typical endodyogeny. Formation of apical complex of daughter merocysts and inner membranous complexes of their pellicle is not accompanied with a nuclear division. Obviously, no connection exists at any point between both nuclei because they are contained on the same stage. Nuclei protract towards the apical ends of future merocysts, where in addition to the bases of the apical complex and inner membranous complex also the outer membrane of the pellicle is formed. This membrane follows a membrane which limits a half-mooned or horseshoe space surrounding the apical end of daughter merocysts (Pl. II, Fig. 1). It is not quite certain whether this space is formed from invaginating parasitophorous vacuole or whether the limiting membrane arises within the meront. The second alternative is more probable because the whole cytoplasm is abundantly vacuolated in this stage of the meront's development. Formation of merocysts without presence of these vesicles was not observed. Merocysts release from the mother cell but they remain connected with it in their posterior part. The outer membranous complex of the pellicle passes from merocysts to mother cell the structure of which has only one surface layer (Pl. II, Fig. 3). Double inner membrane of the merocysts terminates in the place where a merocyst strangulates from mother cell (Pl. II, Fig. 4). At 4 DPI most of first generation merocysts are not separated from mother cell. This fact can clarify the shorter length of first generation meronts at 3 DPI in comparison with the length of meronts at 5 and 6 DPI (see Table 1). Except for being multinucleate, binucleate or trinucleate merocysts do not differ in their structure from typical coccidial merocysts (Pl. III, Fig. 2). They possess a typical developed apical complex, a pellicle with three layers, nuclei with nucleoli, mitochondria, endoplasmatic reticulum and space substances. The inner formation of daughter merocysts has never been observed in multinucleate first generation merocysts which was observed in multinucleate merocysts of *Eimeria magna* (Danforth and Hammond 1972) and in multinucleate third generation merocysts of *E. coeca* described in this paper (see later). Since we did not observe any further development in multinucleate first generation merocysts we presume that they escape as a whole from their host cell and penetrate other epithelial cells of the mucosa of the veriform appendix. Second generation merocysts are formed by eteocorygeny. Formation of their apical complexes and inner membranous complex of their pellicle is connected with the cytoplasmic membrane of a meront (Pl. III, Fig. 3). Merocysts are then shifted into the parasitophorous vacuole whereas the remnant of the mother cell is visible in meronts at this stage both by electron and optical microscope (Pl. III, Figs. 4-5). Subsequently, this remnant of the mother cell disappears. Second generation merocysts are relatively short and thick at first, later they are elongated. Mature merocysts are situated mostly parralel in a meront; their apical complexes are pointed in the same direction (Pl. IV, Fig. 2). The ultrastructure of second generation merocysts is typical of coccidial merocysts; conpious are large numbers of micronemes observed nearly along the whole length of merocysts.

Multinucleate third generation merocysts were also observed by means of TEM (Pl. V, Fig. 2), within which inner membranous complexes and rhoptry anlagen of future merocysts are formed in a close connection with nuclei (Pl. V, Fig. 3). Further
development of these stages was not observed. We presume that a process of inner merozoite formation analogous with observations of Danforth and Hammond (1972) in E. magna takes place here and corresponds to endopolygy.

**DISCUSSION**

Only Cheissin (1947, 1967, 1968) studied endogenous cycle of E. coecolica. However, he did not mention the number of asexual generations. Cheissin recorded meronts measuring 12-18 μm in length which were found in the vermiform appendix of the merozoites measuring 10 × 0.8 μm. In spite of this fact that the merozoite width taken by us was rather greater, we suppose that second asexual generation takes place here. In addition to these stages, Cheissin described stages in the ileum. At 6 DPI he found meronts measuring 12-18 μm which contained 8-15 merozoites. These meronts might correspond to third generation meronts which we observed. Cheissin asserts that endogenous development of E. coecolica takes place in the cecum and vermiform appendix, whereas meronts can be located also in the ileum. We observed all developmental stages in the vermiform appendix or ileum in the present study, never in the cecum. Cheissin's opinion, that all stages are located below the nucleus of the host cell, was supported only as far as gametogony is concerned. Atexual stages of merozoites were also found above the nucleus of the host cell. The prepatent period being 9 days corresponds to Cheissin's description.

First stages of endogenous cycle of E. coecolica were found in our experiments at 4 DPI which is rather late in comparison with other species of rabbit coccidia. We recorded merozoites of E. coecolica in supplementary experiment at 3 DPI in one of the rabbits treated with a dose of 6 million parasites. We observed the only coccidial in small numbers only. No sporozoites of E. coecolica were seen in the two remaining rabbits. Sporozoites were observed in the histological material taken from the epithelium of the vermiform appendix, in which also first generation meronts develop. Other stages except for sporozoites were observed in 3 DPI. Sporozoites were observed in the lymphoepithelial tissue by TEM at 4 DPI. Sporozoites are not usually visible on histological sections of lymphoepithelial tissue as there are large numbers of lymphocytes. Their nuclei stain hard with haematoxylin and overlap sporozoites.

Sporozoites do not reach in a short time after the excystation the epithelial cells of the vermiform appendix, where their further development takes place. It probably causes that the first generation meronts (4 DPI) appear rather late. It is known that free sporozoites of E. magna can be detected in the intestinal lumen as late as at 48 hours post infection (Riley and Robinson 1976). Strenou et al. (1979) observed free or fresh invalid sporozoites of E. perforans which is located in the upper part of the small intestine even at 36 hours post infection.

However, sporozoites need not occur only in the intestinal lumen. It was proved in elamans in chickens (Kogut and Long, 1984; Ferry and Long, 1987) and in rats (Marquardt et al., 1984), that sporozoites do not divide immediately after the excystation, they enter through the intestinal other organs and persist in them. It is possible, even several days post infection to infect with the homogenates obtained from these organs some other hosts in the same manner as they were infected with oocysts, the purpose of such sporozoite migration is not clear. There are also some cases that the process may proceed outside the intestine and S. senegalensis (Heller, 1971). E. fawcensone (Norton et al., 1979). We also observed multineucleate merozoites in E. magna and E. vaculiferous sp. n. (Pakandi, 1988).

Both multineucleate and uninnucleate merozoites have been found in all above species.
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Fig. 1. Oocysts of *E. coccica* (native, ×1200).
Fig. 2. Surface of the mucosa of the vermiform appendix of a control rabbit with dosses (d) (SEM, ×150).
Fig. 3. Sporozoite of *E. coccica* (TEM, ×8500).

Fig. 1. Formation of first generation merozoites (arrow) (TEM, ×15,000).
Fig. 2. First generation merozoite with uninucleate merozoites. Histological section stained with Harris haematoxylin—eosin (HE, ×1300).
Fig. 3. First generation merozoite with uninucleate merozoites (TEM, ×8500).
Fig. 4. Detail of Fig. 3. Inner membraneous complex of merozoites’ pellicle ending in the plane where merozoite strangulates from mother cell (TEM, ×27,000).
Fig. 1. First generation meront with binucleate merozoites (HE, \( \times 1300 \)).

Fig. 2. Binucleate first generation merozoite (TEM, \( \times 10000 \)).

Fig. 3. Initial stage of second generation merozoite formation (TEM, \( \times 8000 \)).

Fig. 4. Second generation meront with the remnant of mother cell (arrow) (HE, \( \times 1500 \)).

Fig. 5. Second generation meront with the remnant of mother cell (TEM, \( \times 9000 \)).

Fig. 1. Second generation meront (HE, \( \times 1500 \)).

Fig. 2. Second generation meront with mature merozoites (TEM, \( \times 8000 \)).

Fig. 3. Second generation merozoites in the slit between dome and mucosa (SEM, \( \times 1700 \)).
Fig. 1. Third generation meront (HE, × 1 600). Fig. 2. Third generation meront. Some merozoites possess more nuclei in the section level (n) (TEM, × 8 100). Fig. 3. Detail of Fig. 2. Apical parts of future merozoite arise in a close connection with nuclei — the inner membranous complex of pellicle (long arrow) and chromatin agglomeration (short arrow) are apparent (TEM, × 33 300). Fig. 4. Fourth generation meronts in the epithelium of ileum (HE, × 1 300).

Fig. 1. Gametogony developing in the epithelium of mucosa of the vermiform appendix (HE, × 300). Fig. 2. Surface of the host cell above the releasing oocyst is without microvillous zone, cytoplasmic membrane of the host cell expands and breaks above the releasing oocyst (SEM, × 1 820).