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Eimeria coecicola Cheissin 1947: endogenous development in gut-associated lymphoid tissue

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Abstract Coccidia-free rabbits were inoculated with different doses of a pure strain of *Eimeria coecicola* and samples of gut were taken at 80, 96, 112, 128, 144, and 160 h postinoculation. The use of a very low infective dose (2–20 oocysts) was sufficient to study the last merogony. The number of merozoites in meronts increased when the infective dose decreased. Only the first merogony of this coccidium in lymphocytes or M-cells of gut-associated lymphoid tissue (GALT) has previously been described. Three other generations of meronts are described herein. All these endogenous stages were observed in the epithelium of the vermiform appendix, *sacculus rotundus*, and Peyer's patches, especially at the bases of the domes. However, in heavily infected tissues the gamonts were seen throughout the epithelium of the GALT. The third- and fourth-generation meronts were of two types. As in other eimerian species of the rabbit, type A meronts produced thick polynucleated merozoites, whereas type B meronts gave rise to large numbers of thin merozoites with one nucleus. Microgamonts were polynucleated and less numerous than macrogamonts. Type A meronts were also polynucleated and less numerous at the end of the merogony. Therefore, types A and B could correspond to a sexual phenotype differentiation occuring during the two asexual phases of multiplication.

Introduction

Eimeria coecicola is a common parasite of domesticated rabbits. This species is considered to be moderately pathogenic (Licois et al. 1992) for rabbit breeding in spite of histopathological changes in the vermiform appendix (Vítovec and Pakandl 1990). Pellérdy (1974) considered *E. coecicola* to be a synonym of *E. neoleporis* Carvalho, 1942, but Cheissin compared the original description of *E. neoleporis* with his own results and confirmed that *E. coecicola* is a valid species. The endogenous development of this coccidium as studied by Pellérdy (1954), Cheissin (1947, 1967, 1968), and Pakandl (1989) involved experiments carried out using conventional rabbits. It was necessary to verify these results in specific pathogen-free (SPF) rabbits. The initial phase of the endogenous cycle of *E. coecicola*, i.e., the migration of sporozoites and merogony in lymphoid cells, has recently been described (Pakandl et al.. 1993). The aim of this paper is to complete the present knowledge of the life cycle of this coccidium.

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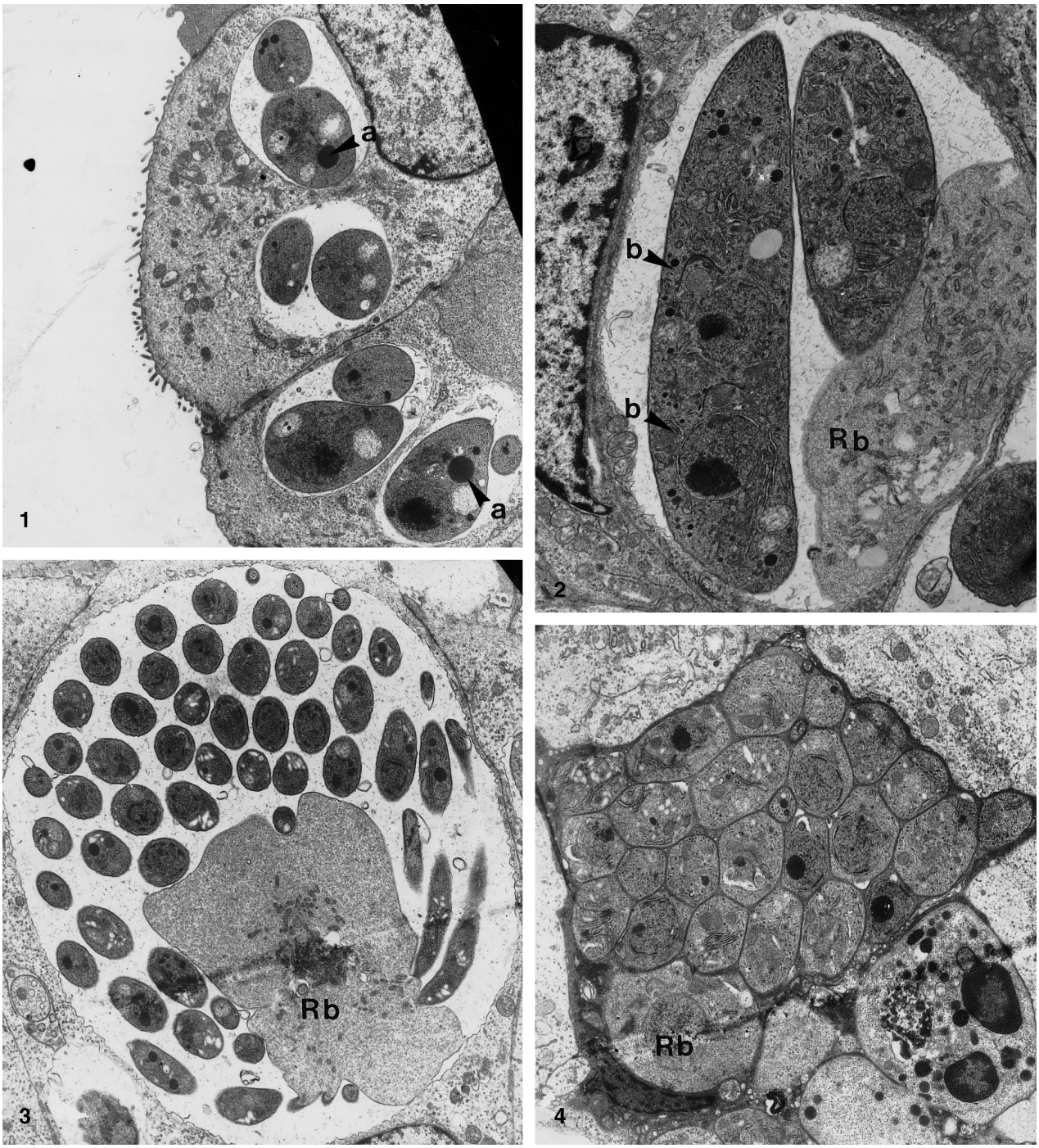
Materials and methods

SPF New Zealand White rabbits aged 5 weeks were used. To synchronize the invasion of host cells, rabbits were inoculated with sporocysts of the pure strain of *Eimeria coecicola* in the duodenum using a previously described method (Pakandl et al.. 1993). Different doses were used for each interval. Euthanasia was performed on inoculated animals at 80, 96, 112, 128, 144, and 160 h postinoculation (p.i.). The infective doses and intervals between inoculation and euthanasia are reported in Table 1. Samples of tissue were taken from the vermiform appendix, *sacculus rotundus*, and Peyer's patches of the ileum. The samples for histology and electron microscopy were processed by previously described methods (Pakandl et al.. 1993). Semithin sections were used to

Table 1 Infective doses of sporocysts of *Eimeria coecicola* used in the present study

		Time to enthanasia (h p.i.)					
		80	96	112	128	144	160
Number of sporocysts	Dose 1	3×10^6	5×10^5	5×10^4	5×10^3	800	800
	Dose 2	3×10^5	5×10^4	5×10^3	5×10^2	80	80
	Dose 3	–	–	–	–	8	8

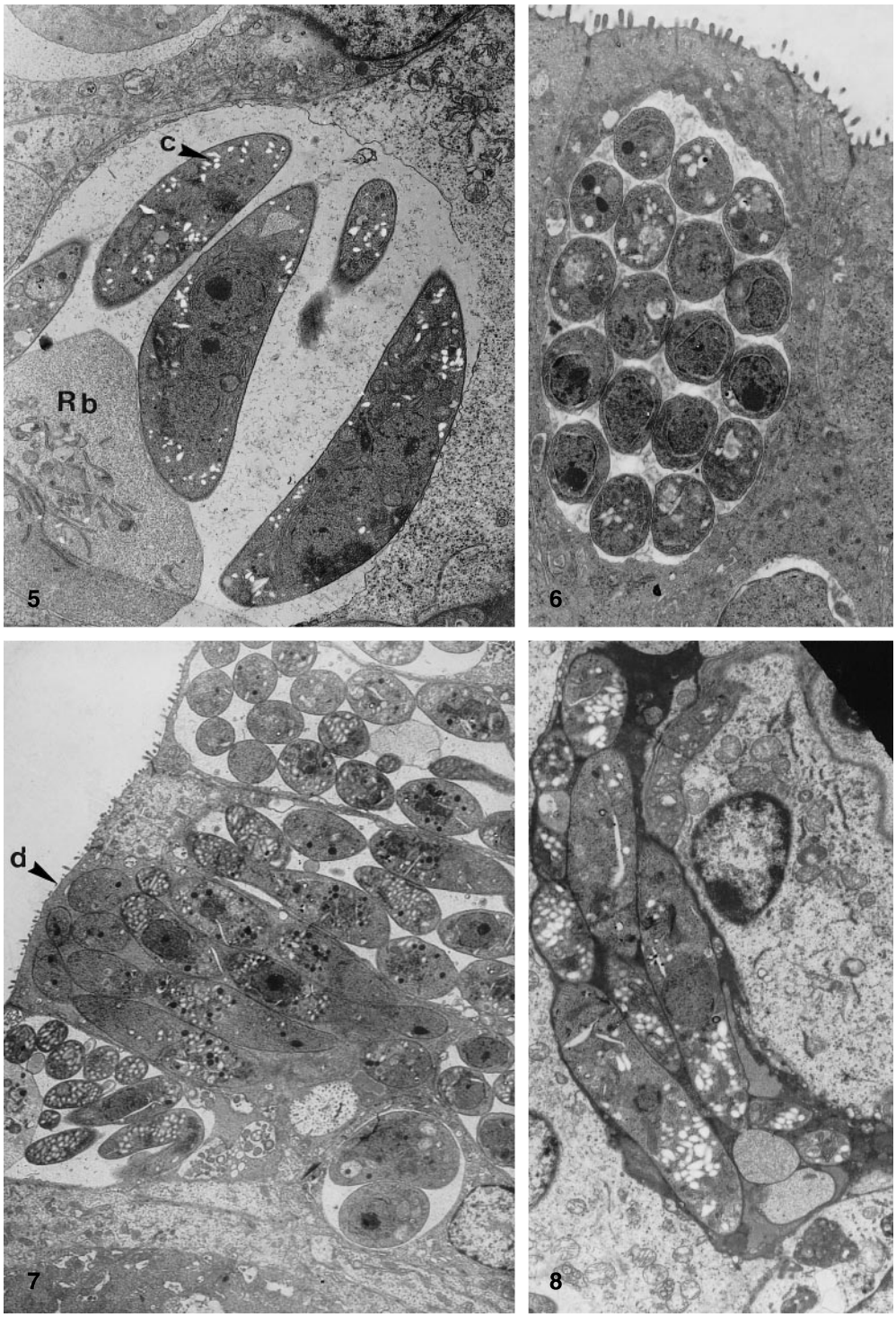


Figs. 1–4 *Eimeria coecicola* meronts, transmission electron micrographs. **Fig. 1** Second-generation meronts. Remnants of refractile body are visible (*arrowheads*, *a*). x4,600. **Fig. 2** Type A meront of the third generation with a residual body (*Rb*). Merozoite anlagen are visible near the two nuclei inside the merozoite (*arrowheads*, *b*). x6,600. **Fig. 3** Type B meront of the third generation producing large numbers of merozoites. x4,600. **Fig. 4** Type B meront inside the M-cell. X4,600 (*Rb* Residual body)

evaluate more precisely the number of merozoites in meronts. Ultrathin sections were examined with a Philips EM 420 electron microscope.

Results

As we have previously observed in the early endogenous stages of *Eimeria coecicola* (Pakandl et al.. 1993), the subsequent development also took place in the vermiform appendix, *sacculus rotundus*, and Peyer's patches (gut-associated lymphoid tissue, GALT). The endoge-



Figs. 5–8 Meronts of the fourth generation. **Fig. 5** Type A meront. The merozoites contain amylopectin granules (*arrowhead*, *c*). x4,600. **Fig. 6** Transversal section of a type B meront. x5,800. **Fig. 7** Type B meronts. The cell in which a parasitophorous vacuole seems to be lacking is probably an M-cell (*arrowhead*, *d*). x4,600. **Fig. 8** Type B meront in an M-cell. X4,600 (*Rb* Residual body)

nous development of the parasite was similar in all these parts of the intestine. Unlike the first merogony, the other merogonies and gamogony took place in the epithelial cells.

Meronts of the second generation with two relatively small but thick merozoites were observed at 80 h p.i. (Fig. 1). Small spherical bodies, probably fragments of the sporozoite refractile body, were often present inside the merozoites. The highest concentration of parasites was detected at the base of the domes. Meronts of this type were always surrounded by a parasitophorous vacuole. We had previously observed the first merogony in lymphocyte-like cells and in the M-cells (Pakandl et al.. 1993) and, therefore, this merogony is the second in the endogenous development of *E. coecicola*. Meronts of this type were also recorded at 96 h p.i.

Third-generation meronts occurred at 96–128 h p.i. and were localized in the epithelium covering the domes

and the fissures around them, especially at the bases of the domes. Unlike the first and second merogonies, there were two types of meronts in the third (and also in the fourth) generation: type A, with a few thick merozoites harboring two or three nuclei, and type B, with a large number of thin merozoites. The type B merozoites arose from ectomerogony. Type A meronts (Fig. 2) had two to ten merozoites with two or (less frequently) three nuclei. Inside these merozoites, early endomerogony was visible even before their separation from the mother cell. The early phase of formation of daughter merozoites was observed. However, it is not clear whether the splitting of daughter merozoites is completed in the same host cell or after penetration into another host cell.

Third-generation type B meronts (Figs. 3, 4) produced 10–200 uninucleated merozoites. The residual body of the meront lay at one pole and merozoites protruded toward the opposite pole of the meront. Mature merozoites were arranged in parallel in the meront and the amount was estimated in the transverse sections. The number of merozoites detected in one meront depended on the intensity of infection of the host tissue, being smaller in massively infected tissues.

A parasitophorous vacuole was present in the epithelial cells harboring both A and B type meronts. These two types were also present in the M-cells, but the parasitophorous vacuole seemed to be absent and merozoites were closely aggregated in the cytoplasm of the host cell (Fig. 4). This phenomenon was also observed in the fourth generation (Figs. 7, 8).

The fourth-generation meronts occurred at 144 and 160 h p.i. Their localization, size, morphology, and number of merozoites were very similar to those of the third generation. Meronts were also segregated in two types: A (Fig. 5) and B (Figs. 6–8). Only two differences were noticed: the fourth-generation merozoites contained more amylopectin granules than the third-generation merozoites, and the ratio of A to B type meronts was different. In the third generation the numbers of both types of meront were approximately equal, whereas in the fourth-generation meronts the type B meronts predominated.

Young gamonts were first observed at 160 h p.i. Generally, they did not differ in their morphology from gamonts of other eimerian species. Their localization depended on the intensity of infection. In rabbits infected by low doses (8 and 80 sporocysts) the localization of gamonts was identical to that seen in the third- and fourth-generation meronts, i.e., in the epithelium covering the domes and the fissures around them. In contrast, in the more heavily infected intestine (800 sporocysts) the epithelium at the bases of the domes proliferated and the gamonts were present in the entire newly formed epithelium.

Discussion

The life cycle of *Eimeria coecicola* was described by Cheissin (1947, 1968) and Pakandl (1989). Cheissin did not establish the number of asexual generations. He described meronts with elongated merozoites in the vermiform appendix. These meronts probably correspond to the B type meronts described in the present paper. Cheissin (1947, 1968) and Pakandl (1989) observed meronts in the epithelium of the ileum, but this was not confirmed in our experiments using SPF rabbits (except for the occurrence of developmental stages in the Peyer's patches). Pellérdy (1954) observed the most distinct pathological changes in the ileocecal valvule and in the vermiform appendix. These findings correspond to the localization of endogenous stages of *E. coecicola*. Pakandl (1989) observed sporozoites in the lymphatic follicles but did not note any merogony in non-epithelial cells. The division of sporozoites in GALT (first merogony) was recently described by Pakandl et al. (1993).

After the second merogony it is impossible to recognize the number of asexual generations in heavily infected rabbits because meronts have a very similar morphology. Using light microscopy, we could observe only the last mature meronts in rabbits infected with eight sporocysts (two oocysts) at 160 h p.i. However, this concentration of parasites was too low for electron microscopy study. Nevertheless, detection by electron microscopy was possible using the infective doses of 80 or 800 sporocysts. We could thus distinguish the third and fourth asexual generations. The inoculation of sporocysts directly into the proximal duodenum is very useful in view of the synchronization of the endogenous development. Moreover, we could clearly observe that if the abundance of parasites in the intestinal mucosa was very high, both the size of the meronts and the number of merozoites occurring in one meront were smaller. We therefore chose samples with the appropriate abundance of parasites from animals infected with different doses.

Certain contradictions between different authors who have described the life cycles of other eimerian species may be explained by variations in the number of merozoites occurring within meronts, depending on the infective dose. This could also partially explain the excretion of oocysts, which is not proportional to the infective dose (Coudert 1989; Licois et al. 1992).

Polynucleated merozoites have been observed in almost all known species of rabbit coccidia. It is very probable that these type A merozoites become the meronts of the next generation, also producing type A polynucleated merozoites. The decreasing number of type A meronts occurring in the following generations can easily be explained by the smaller number of type A meronts. This is in agreement with the observation that microgamonts are obviously less numerous than macrogamonts. Meronts of rabbit coccidia are therefore very probably sexually determined: type A meronts may be male and type B meronts, female. This hypothesis was first suggested by Streun et al.. (1979) and has been confirmed de facto by all life-cycle studies of rabbit *Eimeria*. In the present work, all the second-generation merozoites were uninucleated, whereas the third- and fourth-generation meronts were of two types. Thus, the genetic variation is phenotypically ex-

pressed from the third generation; this is unusual in rabbit coccidia because in all other species, two types of meronts can be distinguished as early as in the first generation. It is also unusual that the first generation develops in nonepithelial cells (Pakandl et al.. 1993).

Localization of all endogenous stages in epithelial cells showed that meronts and gamonts of *E. coecicola* preferred very young cells at the bases of domes for development. Nevertheless, in heavily parasitized animals, proliferation of the epithelium took place and the gamonts were present in the entire epithelium, including the superficial epithelium of the mucosa, in which meronts were never seen. This type of proliferation of the epithelium has previously been seen only in the bile ducts of rabbits infected with *E. stiedai* (Pellérdy and Dürr 1970).

The absence of a parasitophorous vacuole in infected M-cells is surprising. In our previous work (Pakandl et al. 1993) we were incapable of recognizing a parasitophorous vacuole around the meronts, even at a high magnification. We do not believe that this was due to an artefact such as poor fixation, because the other cellular structures were well preserved. The unusual localization of the endogenous stages of this coccidium in three different parts of the intestine (vermiform appendix, *sacculus rotundus*, Peyer's patches) probably results from the initial development of this species in the lymphoid cells of the GALT. Thus, this coccidium may be a very interesting model for further studies.

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