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Life cycle of *Eimeria vejdovskyi* Pakandl, 1988: electron microscopy study

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Abstract The endogenous development of the rabbit coccidium *Eimeria vejdovskyi* was studied in SPF rabbits using light and electron microscopy. All endogenous stages were seen in the ileal epithelium. There were two types of meronts in each of five asexual generations. Type A produced fat, polynucleate merozoites while type B meronts had slender uninucleate merozoites. First- second- and third-generation meronts were in the crypts. The first meronts were at the bottom of the crypts, very often in the Paneth cells. The meronts of the fourth generation were in the middle of the villi; the fifth-generation meronts were recorded in the middle and at the top of the villi. The prepatent period was 10 days.

Introduction

A new *Eimeria* species was recently identified and its life cycle partly described by Pakandl (1988) using light microscopy in conventional rabbits pretreated with an anticoccidicum. More recently, the validity of the species *Eimeria vejdovskyi* was supported by Ceré et al. (1995) who used random amplified polymorphic DNA to study inter- and intraspecific variation among rabbit coccidia. We studied the endogenous development of *E. vejdovskyi* in SPF rabbits using histology and transmission electron microscopy.

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Materials and methods

Six-week-old New Zealand White rabbits originating from a coccidia-free breeding rabbitry (Coudert et al. 1988) were used.

A pure strain was constituted using the techniques described by Coudert et al. (1979). Ten oocysts were isolated from field samples (Pakandl 1988). Single oocysts were inoculated in coccidia-free rabbits and ten rabbits were individually checked after inoculation. Oocyst size, sporulation time and prepatent period were identical. The ten isolates were therefore considered to belong to the same species and mixed together to constitute a pure strain. Animals were inoculated with sporocysts directly into the duodenum as previously described (Pakandl et al. 1993). The doses of sporocysts used are summarized in the Table 1.

Rabbits were sacrificed every 18 h up to 198 h postinoculation (p.i.) by an overdose of pentobarbital. The feces of two other groups inoculated with 10^3 and 10^5 sporocysts were collected daily to record the start of oocyst output.

Tissue samples were taken from the duodenum, jejunum, ileum (one sample 15 cm and the second 5 cm before the valvula ileocaecalis), cecum, and colon. The samples were processed for electron microscopy by fixation with 3% glutaraldehyde in 0.1 *M* cacodylate buffer, postfixation with osmium tetroxide and embedding in Spurr as described by Streun et al. (1979). Semithin sections were stained with Warmke's polychrome for light microscopy, and ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Philips EM 420 electron microscope.

Scrapings were taken from the same sites as above, fixed with methanol, stained with Giemsa and used to count the nuclei in the merozoites.

Results

All endogenous stages of *E. vejdovskyi* were seen exclusively in the ileal epithelium. Five generations of meronts developed at different intervals (Table 2). The meronts of each generation were of two types. Type A meronts formed a smaller number of larger merozoites with several nuclei. Early formation of daughter merozoites were visible inside these meronts. Whereas endomerogony is characteristic for the type A meronts, the type B meronts gave rise to larger numbers of uninucleate merozoites by ectomerogony. The ratio of A- to B-type meronts progressively decreased from the first to

Table 1 Experimental design: doses of sporocysts given in duodenum

	Time examined (p.i. postinoculation)							
	18–72	90	108	126	144	162	180	198
Initial dose	5×10^7	5×10^6	10^5	10^5	10^4	10^4	10^3	10^3 or 10^2

the fifth generation. The endogenous development of *E. vejdovskyi* is described more fully below.

No parasite stages were found 18 h p.i. At 36 h p.i., the sporozoites were found at the bottom of crypts, very often in Paneth cells and usually with one nucleus. Two or more nuclei were seldom present in a sporozoite, suggesting the beginning of merogony.

First-generation meronts were seen 54 and 72 h p.i. Two types could be distinguished. The first (type A) was a sporozoite-shaped meront resembling a sporozoite (Fig. 1). However, several nuclei could be seen inside them and initial phases of endomerogony were noted. Near the nuclei, i.e., inside the cell, the apical ends of daughter merozoites were sometimes observed with an inner membranous complex of the pellicle, conoid, and rhoptry anlage. Type B meronts (Fig. 2) produced about 20–50 merozoites. The apical ends of daughter merozoites were formed in close proximity to the parent pellicle and later the merozoites protruded into the parasitophorous vacuole. Type B meronts thus underwent ectomerogony. Remains of the refractive body of sporozoites were visible inside the merozoites.

From 72 to 108 h p.i., second-generation meronts were found in a similar localization to the preceding stages. Meronts of both types formed two to four or, less frequently, up to eight nearly spherical merozoites. Type A merozoites (Fig. 3) contained two nuclei whereas the type B merozoites (Fig. 4) were uninucleate.

Third-generation meronts (Fig. 5) were recorded 108 and 126 h p.i; the meronts also developed in the crypts, but not only at the bottom. They also inhabited epithelial cells in a slightly more distal area of the mucosa. While the second-generation merozoites were nearly spherical in shape, the merozoites of the last three generations were elongated. Type A meronts produced 4–20 fat merozoites harboring two or, less frequently, three nuclei. Type B meronts formed 10–40 smaller uninucleate merozoites.

Fourth-generation meronts (Fig. 6) were observed in the wall of the intestinal villi 126, 144, and 162 h p.i. The meronts of this generation were the largest in the endogenous development of this coccidium. They gave rise to approximately 25–70 (type A) and 50–200 merozoites (type B). The merozoites of both types protruded in all directions from the center of the meront. Type A merozoites contained two nuclei, while numerous slender merozoites of type B had one nucleus.

Fifth-generation meronts were seen 162 and 180 h p.i. and were also present in small numbers as late as 198 h p.i. Meronts of this generation were located in the upper part of the villi. Type A (Fig. 7) formed 2–4 merozoites with two or occasionally three nuclei, whereas type B meronts (Fig. 8) produced 4–20 merozoites with one nucleus. Type A meronts were much less frequent in this generation than type B meronts.

Young gamonts were first recorded 198 h p.i. in a similar localization to the last-generation meronts. No peculiarities were noted in their morphology and ultrastructure compared with other eimerians and therefore no detailed description of gamonts is given.

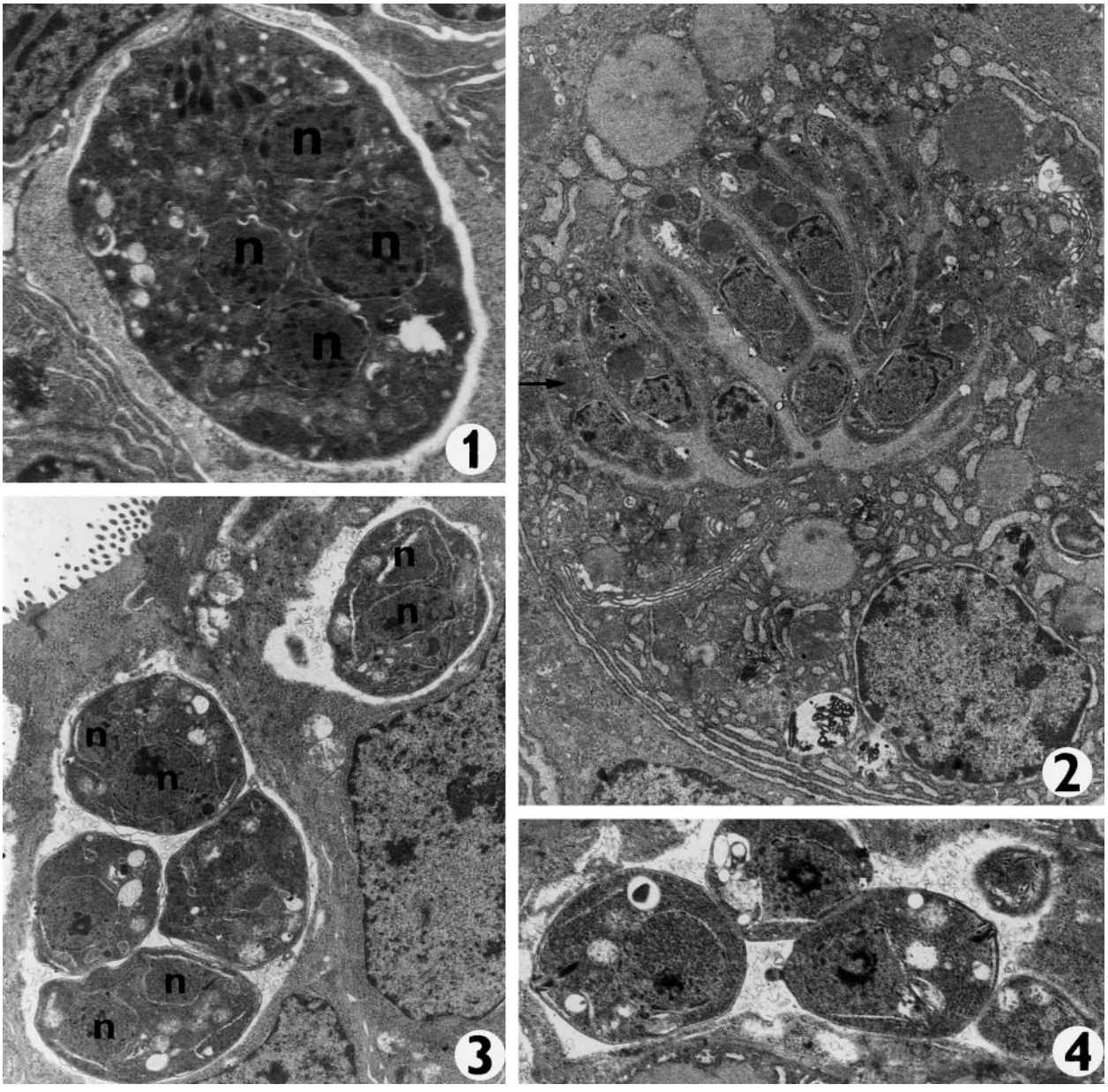
A prepatent period of 10 days was established in the infected control animals.

Discussion

Most rabbit *Eimeria* are relatively easy to identify. Nevertheless, three have very similar morphological features: *E. media* Kessel, 1929, *E. coecicola* Cheissin, 1947, and *E. vejdovskyi* Pakandl, 1988. The similar appearance and oocyst size have resulted in some confusing descriptions. Pellérdy and Babos (1953) called "*E. media*" a coccidium from the vermiform appendix which is in fact *E. coecicola*. Pakandl (1988) identified two species with similar oocyst morphology: one with larger oocysts and a 10-day prepatent period, the second with smaller oocysts and a 5-day prepatent period. Pakandl reserved the name *E. media* for the first because the oocyst size corresponded to the description given by Kessel (1929) and Kessel and Jankiewicz (1931). Pakandl (1988) called the second species *E. vejdovskyi*

Table 2 Succession of generations in the endogenous development of *Eimeria vejdovskyi* (*p.i.* Postincoculation)

hp.i.	Coccidian stages	Localization	Fig.
18	Nothing found		
36	Sporozoites, exceptionally polynucleate	Bottom of the crypts	
54	First-generation meronts	Bottom of the crypts	1, 2
72	First- and second-generation meronts		
90	Second-generation meronts	Crypts	3, 4
108	Second- and third-generation meronts	Crypts	
126	Third- and fourth-generation meronts		5
144	Fourth-generation meronts	Wall of the villi	6
162	Fourth- and fifth-generation meronts		
180	Fifth-generation meronts	Upper part of the villi	7, 8
198	Young gamonts	Upper part of the villi	

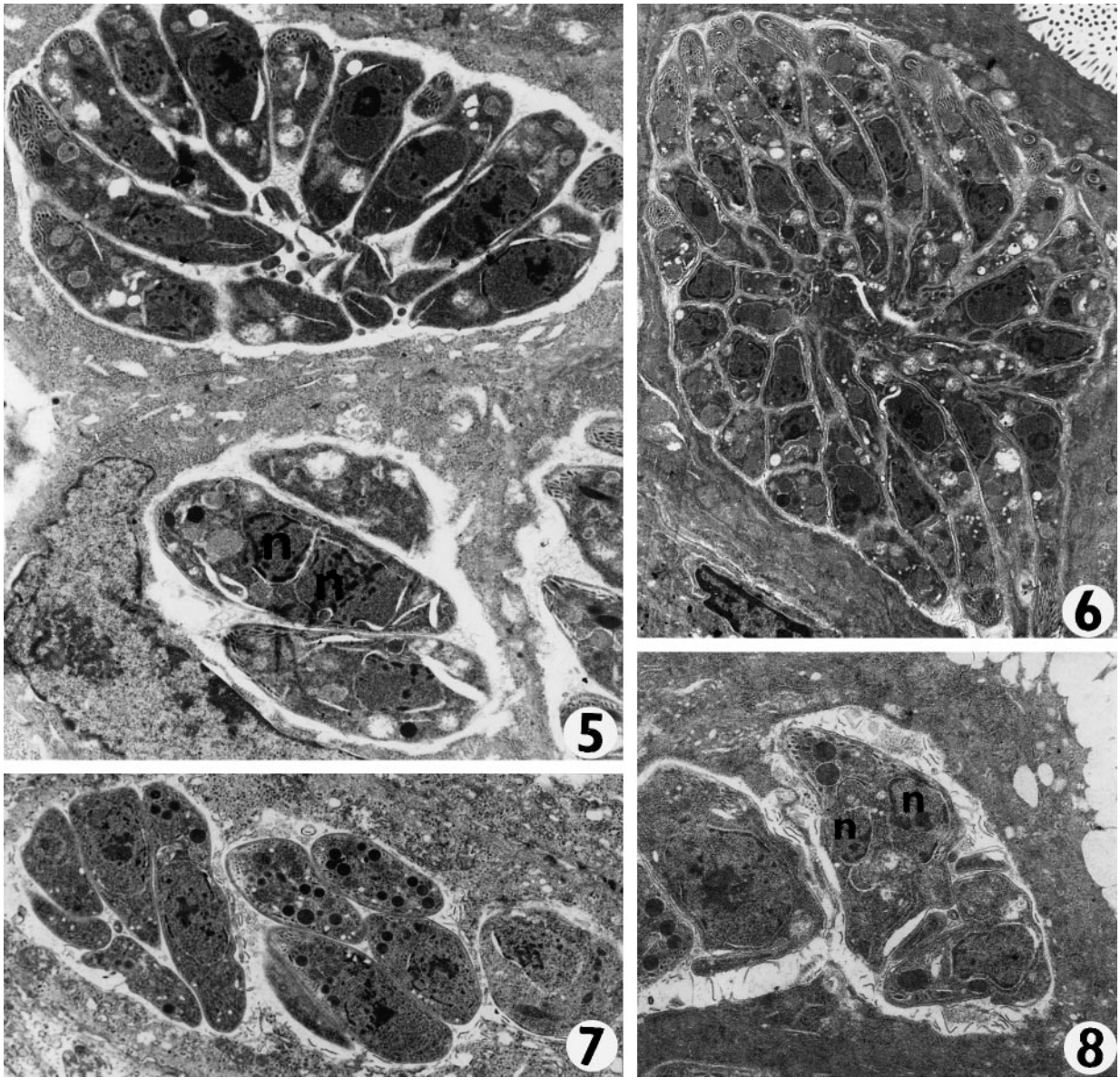


Figs. 1–4 *Eimeria vejdovskyi* meronts, transmission electron micrographs
Fig. 1 First-generation type A meront (sporozoite-shaped meront). Four nuclei (*n*) are visible (× 7,500)
Fig. 2 First-generation type B meront in Paneth cell. Note the remnants of the refractive body of the sporozoite (*arrow*) (× 7,700)
Fig. 3 Second-generation type A meronts; two nuclei (*n*) are visible in several merozoites (× 6,300)
Fig. 4 Second-generation type B meront with round merozoites (× 7,700)

sp. n. However, all other researchers use the name *E. media* for the species with smaller oocysts and 5-day prepatent period. For this reason, we propose to change the names given by Pakandl (1988) as follows: to retain *E. media* for species having smaller oocysts and a 5-day prepatent period and to use *E. vejdovskyi* for the other species with larger oocysts and a 10-day prepatent period.

The life cycle of *E. vejdovskyi* was first described by Pakandl (1988) in conventional rabbits pre-treated with sulfonamides and using light microscopy. Detailed study of the endogenous cycle of *E. vejdovskyi* presented in this paper revealed two additional generations – the first and the second. Thus, the three generations described by Pakandl (1988) correspond to the third, fourth, and fifth generations respectively. The morphology and localization of these meronts and gamonts are in complete agreement with the previous description given by Pakandl (1988).

The 10-day prepatent period is the longest among rabbit intestinal coccidia (Coudert et al. 1995) and this is probably related to the large number of asexual generations. Besides *E. vejdovskyi*, five merogonies have been observed only in *E. flavescens* (Norton et al. 1979), a species also exhibiting a long prepatent period (9 days).



Figs. 5–8 Meronts of three successive generations
Fig. 5 Third-generation meronts, type B (*above*) and A (*below*). Two nuclei (*n*) are visible in the merozoites of the type A meront (× 7,500)
Fig. 6 Fourth-generation type B meront with numerous uninucleated merozoites (× 5,200)
Fig. 7 Fifth-generation type B meronts (× 7,900)
Fig. 8 Fifth-generation type A meront (× 8,300)

We recorded sporozoites in the ileal crypts 36 h p.i., mostly without any indication of starting merogony. However, we were unable to find them 18 h p.i. in the ileum, duodenum, or jejunum. Although the site of entry of sporozoites is unknown, it seems probable that, as in other rabbit species, the sporozoites enter the intestine in the duodenum and upper jejunum and then migrate by a route which remains unclear. This phenomenon was observed in *E. intestinalis* (Drouet-Viard et al. 1994), *E. coecicola* (Pakandl et al. 1993) and *E. magna* (Pakandl et al. 1995).

The identification of the five generations was relatively easy because they differed in their localization and morphology. Moreover, the method of inoculation of animals with sporocysts into the duodenum is valuable for synchronization of endogenous development. As the coccidium multiplies in the host, different sporocyst dosages are necessary to study the early or late stage. A very small infective dose (10^2 sporocysts) is sufficient to observe gamonts. Only meronts of the fifth generation could be seen after inoculation of animals with 10^3 sporocysts and this also enabled us to distinguish the last generation.

Polynucleated merozoites have been described in most rabbit coccidia (Pellérdy and Dürr 1970; Ryley and Robinson 1976; Norton et al. 1979; Streun et al. 1979; Pakandl 1988; Licois et al. 1992; Pakandl et al. 1996a, 1996b, 1996c), although this is not common in coccidian species from other hosts. Streun et al. (1979) were the first to articulate a theory that the two types of meronts

and merozoites precede macro- or microgamonts, respectively.

As the apical complex, pellicle, and other organelles do not differ in uni- and polynucleate merozoites, we believe that the polynucleate merozoites can leave the host cell and penetrate into another one. If this is true, young meronts of the next generations are merozoite-shaped schizonts and, as in the subsequent generations, the young first-generation meronts are sporozoite-shaped schizonts. Unfortunately, this cannot be easily proved in vivo. Although we could not calculate the ratio of type A to type B meronts exactly, type A numbers decreased in following generations, apparently due to smaller numbers of merozoites in the type A meronts, and in the last generation, type A was much less frequent. This corresponds to a small number of micro-compared with macrogamonts. It seems probable that type A and B meronts of *E. vejdovskyi* and other rabbit *Eimeria* species are sexually determined and, unlike coccidian species from other hosts, this is also expressed in their morphology.

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