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Original article

***Eimeria media* Kessel 1929: comparative study
of endogenous development between precocious
and parental strains**

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Summary — Endogenous development of a pure strain of *Eimeria media* and of a precocious line derived from this strain was studied in specific pathogen-free (SPF) rabbits. Endogenous development of the parental strain comprised three generations and the gamogony began 76 h post-inoculation (pi). Two types of meronts were observed in each generation. The type A meronts gave rise to large, polynucleated merozoites present in low numbers. Multiplication was carried out by endomerogony. Within type B meronts, merozoites arose from ectomerogony. These were slender and more numerous than those of type A. The sporozoite refractile body was divided and distributed into the first and second generation merozoites but not into the third. The endogenous development of the precocious line was similar to that of the parental strain except that no refractile body was observed and the last merogony was absent. Gamogony appeared 60 h pi.

rabbit / coccidia / endogenous development / precocious line / *Eimeria media*

Résumé — *Eimeria media* Kessel 1929 : étude comparative du développement endogène des souches précoce et parentale. L'étude comparative du cycle d'une souche pure d'*Eimeria media* et d'une lignée précoce qui en dérive a été réalisée chez des lapins SPF. Le cycle interne de la souche parentale comportait trois générations de mérontes et la gamogonie a commencé 76 heures après l'inoculation. Deux types de mérontes ont été observés dans chacune des générations. Les mérontes de type A contenaient de gros mérozoïtes polynucléés peu nombreux. La multiplication s'est effectuée par endomérogonie. Dans les mérontes de type B, les mérozoïtes résultaient d'une ectomérogonie. Ils

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étaient fins, uninucléés et plus nombreux que ceux des mérontes de type A. Le globule réfringent du sporozoïte s'est divisé et s'est retrouvé dans les mérozoïtes de première puis de deuxième génération mais pas dans ceux de troisième génération. Le cycle interne de la lignée précoce était similaire à celui de la souche parentale mais on a noté l'absence totale de globule réfringent et la troisième mérogonie était absente. La gamogonie est apparue 60 heures après l'inoculation.

lapin / coccidie / cycle / souche précoce / Eimeria media

INTRODUCTION

The life cycle of *Eimeria media*, one of the most common rabbit parasites, has been described by Rutherford (1943), Pellérdy and Babos (1953), Pellérdy (1974), Cheissin (1967) and recently by Pakandl (1988). However, all these observations were only made on conventional rabbits and using light microscopy. Because many field isolates of coccidia are resistant to anticoccidial drugs, attenuated lines of coccidia represent a very promising possibility for the prevention of coccidiosis. Some precocious lines of rabbit coccidia were obtained in the Laboratoire INRA de pathologie du lapin in Tours: *E. intestinalis* (Licois et al, 1990), *E. media* (Licois et al, 1994), *E. magna* (Licois et al, 1995). For *E. media* the prepatent period was reduced from 108 h with the parental strain to 72 h with the precocious line. The main aim of the present paper was to describe the life cycle of *E.*

media in specific pathogen free (SPF) rabbits and to compare endogenous development of the parental and precocious strains of this coccidium.

MATERIALS AND METHODS

Six-week-old Californian White rabbits originating from a coccidia-free breeding rabbitry (Coudert et al, 1988) were used. A pure strain of *E. media* and a precocious line derived from this strain (Licois et al, 1994) were used. In order to synchronize the invasion of host cells, rabbits were inoculated with sporocysts directly into the proximal duodenum as described earlier (Pakandl et al, 1993). Euthanasia was performed by an overdose of pentobarbital 24, 40, 60, 76 or 96 hours post inoculation (h pi). Due to the lower multiplication rate of precocious lines, the doses of sporocysts given for studying the beginning of their life cycle were higher (table I).

Tissue samples were taken from the duodenum, proximal and middle jejunum and the ileum. They were fixed with 5% glutaraldehyde in 0.2 M cacodylate buffer, postfixed by osmium tetroxide

Table I. Infective doses of sporocysts of *E. media*.

	Times of tissue sampling (h pi) ^a				
	24	40	60	76	96
Parental strain	1.5 x 10 ⁷	1 x 10 ⁷	5 x 10 ⁴	5 x 10 ⁴	5 x 10 ²
Precocious line	1.5 x 10 ⁷	1 x 10 ⁷	5 x 10 ⁵	5 x 10 ⁵	—

^a Hours post inoculation.

and embedded in Spurr as described by Streun et al (1979). Semi-thin sections were stained with Warmke's polychrome for light microscopy. Ultra-thin sections were contrasted by uranyl acetate and lead citrate and examined with a Philips EM 420 electron microscope (Pakandl et al, 1993).

RESULTS

The entire development of both the parental and precocious strains took place in the duodenum, jejunum and, with a paucity of developmental stages, in the ileum. All developmental stages were located in the walls and tips of the villi. We did not observe any developmental stage in the crypts.

24 h pi

Very young polynucleated meronts were observed for both strains. Some characteristics of the sporozoite were retained (fig 1). An apical complex and a pellicle were present. The refractile body was divided into several smaller bodies in the parental strain, whereas it was absent in the precocious line. Some 'merozoite anlage' were seen inside the meronts and this indicated early endomerogony. Meronts of this type are usually described as type A. Type B meronts with eight to twenty mononucleated merozoites (fig 4) were also observed in both strains. These first generation meronts were characterized by the persistence of a large refractile body inside each merozoite. No refractile body was seen in the precocious line (fig 5).

40 h pi

Mature meronts occurred in both parental and precocious strains. Two types of meronts were observed: type A with two to six larger merozoites harbouring two, or

rarely three nuclei (fig 2), and type B which gave rise to five to twenty mononucleated merozoites (fig 6). The type B meronts differed in their morphology from those observed at 24 h pi, because the refractile bodies of the merozoites were absent or very small. These type B meronts could be of a second generation. We did not notice any conspicuous difference in the size of meronts or number of merozoites in each meront between the two strains.

60 h pi

In the parental strain, young third generation meronts (fig 7) were the most common stage at this time. First gamonts were seen in the precocious line (fig 9).

76 h pi

In the precocious line, the last meronts of the second generation and the first young gamonts were seen. In the parental strain, mature third generation meronts were observed at this time. As in the other generations, they were of two types: type A containing two to six large merozoites harbouring four to eight nuclei (fig 3) and type B producing ten to forty smaller uninucleated merozoites (fig 8). The difference in size between A- and B-type merozoites was more conspicuous than in the second generation. Similarly, as in the first asexual generation of both strains, the uninucleated merozoites arose from ectomerogony, whereas we observed endomerogony in the polynucleated merozoites. No refractile bodies were seen in the third generation meronts.

96 h pi

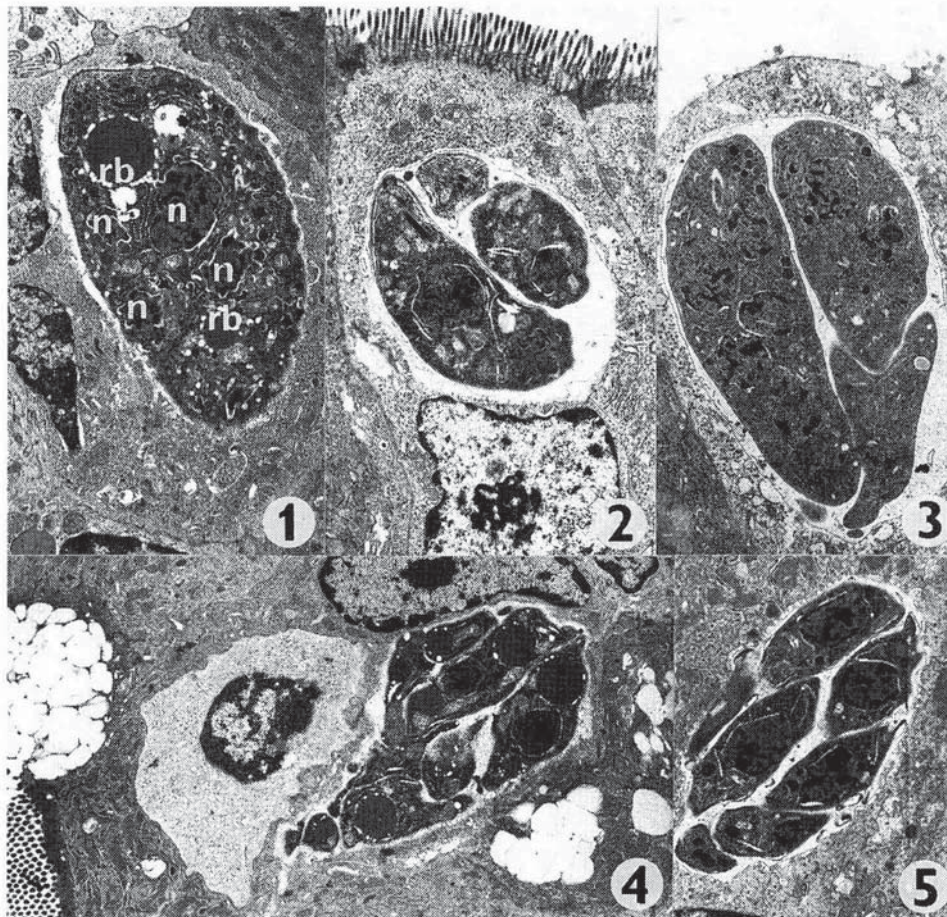
Gamogony occurred in the parental strain.

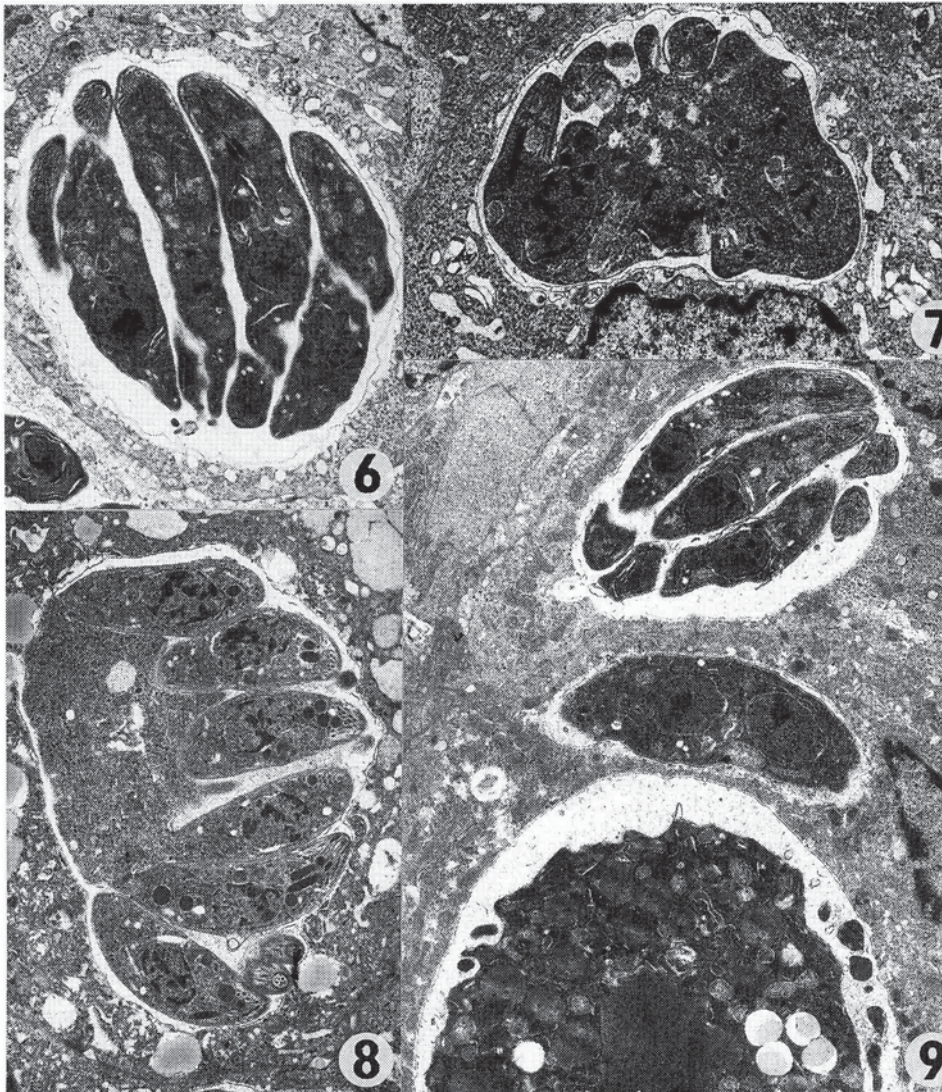
DISCUSSION

E media is not easy to study, even in heavily infected animals, because the parasitized tissues are disseminated in small areas along 2–3 m of the small intestine. Furthermore there are no obvious specific macroscopic lesions. The literature on the endogenous development of this species is summarized in table II.

The endogenous development of *E media* was first described by Rutherford (1943). This author probably supposed a similar development in all four rabbit coc-

cidia known at this time (*E irresidua*, *E magna*, *E media*, *E perforans*). He described two asexual generations with two types of meront in each generation, even in *E media*. Taking into consideration the prepatent period (9 days!), his description is doubtful. Pellérdy and Babos (1953) also described two asexual generations with two types of meront for both generations in the endogenous development of this coccidium. However, their experimental rabbits were probably contaminated with another coccidian species, because these authors found severe lesions in the large intestine. The localization of *E media* in the large intestine





Figs 1–9. Transmission electron micrographs of the endogenous stages of the parental (figs 1–4, 6–8) and the precocious (figs 5, 9) strains of *E. media*. x 4 600.

Fig 1. Meront of the first generation, type A, 24 h pi, with several nuclei (n) and refractile bodies (rb).

Fig 2. Type A meront, 40 h pi, with binucleated merozoites.

Fig 3. Meront of the last type A generation with polynucleated merozoites.

Fig 4. Type B meront, first generation, 24 h pi.

Fig 5. Type B meront, first generation, 24 h pi.

Fig 6. Second generation meront, type B, 40 h pi.

Fig 7. Young meront of the third generation, type B, 60 h pi.

Fig 8. Third generation meront, type B, 76 h pi.

Fig 9. Meronts of type B (above), type A (in the middle) and young macrogamonts (below), 60 h pi.

Table II. Summary of the data on endogenous development of *E. media* obtained from different works.

Reference	1st merogony		2nd merogony		3rd merogony		Prepatent period (days)
	Time (days)	Type	Time (days)	Type	Time (days)	Type	
		A ^a B	A B	A B	A B	A B	
Kessel, 1929							
		first description of the species					
Rutherford, 1943	4	2-10	12-36	6	2-10	24-36	8-9
Cheissin, 1967	2-3	6-11		4	2-18	4-6	35-130
Pakandl, 1988	?	?	?	2	2-8	7-25	3-5
This paper: parental strain	24 h	none	8-20	40 h	2-6	10-20	76 h
This paper: precocious line	24 h	none	8-20	40 h	2-6	10-20	2-6
							15-45
							10-40
							2.5 ^b

^a Number of merozoites in A- or B-type; ^b presence of mature oocysts in caecal content.

has never been confirmed (Cheissin, 1967; Pakandl, 1988; Coudert, 1989; Coudert et al, 1995 and the present paper). The description of the life cycle of *E media* given by Pakandl (ie, *E vej dovskyi* according to Pakandl, 1988), corresponds to the last two generations described in this paper. Pakandl did not observe first merogony, probably because he used infective doses which were too low or partially-immunized rabbits.

The life cycle of *E media* is characterized by a diffuse localization throughout most of the small intestine and by the presence of meronts with relatively few merozoites (maximum 40). A- and B-type meronts are very probably sexually determined and, as supposed by Streun et al (1979), type A meronts seem to be male and type B female.

The number of asexual generations for type A meronts is not clear. For both strains we suppose that type A meronts seen 24 and 40 h pi were of the same generation. This would be the only generation for the precocious line. The following generation of the parental strain occurred 60 h pi (young meronts) and later (76 h pi). This generation is characterized by a large number of nuclei in each merozoite.

We identified two generations of B-type meronts in the precocious line and three in the parental strain. Each had a particular morphology. In the parental strain, all first-generation merozoites contained a large refractile body and the third-generation meronts produced larger numbers of merozoites.

The precocious line of *E media* was characterized by a similar lifespan of the generation to that of the parental strain but the last merogony was lacking. In the case of *E intestinalis*, among the four generations described for the parental strain (Licois et al, 1992), the third is lacking in the precocious line (Licois et al, 1989). Jeffers (1986) stated that the shortened life cycle in poultry could be due to a reduction in the num-

ber of merogonies, or to a reduction in the lifespan of one or several generations. As for *E media*, no difference in the lifespan of the merogonies was seen between the precocious line and the parental strain of *E intestinalis* (Licois et al, 1989). Another feature of the precocious line of *E media* was the complete absence of the refractile body and this corresponds to the particular morphology of the oocysts in all precocious lines of rabbit coccidia.

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REFERENCES

- Cheissin EM (1967) *Life Cycles of Coccidia of Domestic Animals*. Public House Nauka, Leningrad [in Russian]
- Coudert P (1989) Some peculiarities of rabbit coccidiosis. In: *Proceedings of the Vth International coccidiosis conference: Coccidia and intestinal coccidiomorphs* (S Holdas, ed), October 17–20, Tours, France, Les colloques de l'Inra, Paris, 49, 481-488
- Coudert P, Licois D, Besnard J (1988) Establishment of a SPF breeding colony without hysterectomy and handrearing procedures. In: *Proceedings of the 4th congress of the World Rabbit Science Association* (S Holdas, ed), October 10–14, Budapest, RCAPN, Herceghalom, Hungary, 137-148
- Coudert P, Licois D, Drouet-Viard F (1995) *Eimeria* species and strains of rabbit. In: *Guidelines on Techniques in Coccidiosis Research* (J Eckert, R Braun, MW Shirley, P Coudert, eds) Luxembourg Office Official Publications, European Communities, 52-73
- Jeffers TK (1986) Attenuation of coccidia. A review. In: *Proceedings of the Georgia Coccidiosis Conference* Nov 19–21, Atlanta (R McDougald, P Joyner, PL Long, eds), pp 482-497
- Kessel JF (1929) The *Eimeria* of domestic rabbits. *J Parasitol* 16, 100
- Licois D, Coudert P, Bahagia S (1989) Some biological characteristics of a precocious line of *E intestinalis*. In: *Proceedings of the Vth International Coccidiosis Conference: Coccidia and intestinal coccidiomorphs*.

- (P Yvoré, ed), October 17–20, Tours, France, Les colloques de l'Inra, Paris 49, 503-508
- Licois D, Coudert P, Boivin M, Drouet-Viard F, Provôt F (1990) Selection and characterization of a precocious line of *Eimeria intestinalis*, an intestinal rabbit coccidium. *Parasitol Res* 76, 192-198
- Licois D, Coudert P, Bahagia S, Rossi GL (1992) Endogenous development of *Eimeria intestinalis* in the rabbit (*Oryctolagus cuniculus*). *J Parasitol* 78, 1041-1048
- Licois D, Coudert P, Drouet-Viard F, Boivin M (1994) *Eimeria media*: Selection and characterization of a precocious line. *Parasitol Res* 80, 48-52
- Licois D, Coudert P, Drouet-Viard F, Boivin M, (1995) *Eimeria magna*: Pathogenicity, immunogenicity and selection of a precocious line. *Vet Parasitol* 60, 27-35
- Pakandl M (1988) Description of *Eimeria vejnovskyi* sp n and redescription of *Eimeria media* Kessel, 1929 from the rabbit. *Folia Parasitol (Prague)* 3, 1-9
- Pakandl M, Coudert P, Licois D (1993) Migration of sporozoites and merogony of *Eimeria coecicola* in gut-associated lymphoid tissue. *Parasitol Res* 79, 593-598
- Pellérdy LP (1974) *Coccidia and Coccidiosis*. Akademiai Kiado, Budapest
- Pellérdy LP, Babos (1953) Untersuchungen über die endogene Entwicklung sowie pathologische Bedeutung von *Eimeria media*. *Acta Vet Acad Sci Hung* 3, 173-178
- Rutherford RL (1943) The life cycle of four intestinal coccidia of the domestic rabbit. *J Parasitol* 29, 10-32
- Streun A, Coudert P, Rossi GL (1979) Characterization of *Eimeria* species. II. Sequential morphologic study of the endogenous cycle of *Eimeria perforans* (Leuckart, 1879; Sluiter and Swellengrebel, 1912) in experimentally infected rabbits. *Z Parasitenkd* 60, 37-53