Selection and characterization of a precocious line of *Eimeria intestinalis*, an intestinal rabbit coccidium

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**Abstract.** A precocious line of *Eimeria intestinalis* was obtained by selection for early development of oocysts in rabbits and after six consecutive passages in animals. This line (EiP) was derived from a wild strain (EiO) isolated in 1975 from the caecal content of a rabbit with coccidiosis. The prepatent period of the EiP strain was reduced from 215 h to <144 h, the result being that the oocyst sporulation time was the same for both lines. The excreted and unsporulated oocysts had exactly the same shape, but microscopical examination of the sporulated oocysts showed a marked difference between EiP and EiO strains. A huge refractile globule was located in each of two sporocysts of the precocious line, whereas no refractile globule was seen in the other two. The EiP line had a reproductive potential much lower (1000 times) than that of its parent strain EiO and, as judged by the weight gain, mortality and lesions that also occurred in the jejunum and above all in the ileum, its pathogenicity was substantially reduced.

In spite of the many studies carried out on poultry, no attempt to immunize chickens with killed coccidia or material derived from these parasites has to date protected the animals from subsequent infection. Only inoculation with live coccidia provides protection (Long and Rose 1982; Rose 1982, 1986; Rose and Long 1980). In addition, the use of *Coccidia* strains with attenuated pathogenicity has been the object of several studies (Jeffers 1975; Johnson et al. 1986; McDonald and Ballingall 1983a, b; McDonald et al. 1982, 1986; Shirley et al. 1984; Shirley and Bellatti 1984).

In the rabbit, *Eimeria intestinalis* is without doubt one of the most pathogenic coccidia (Catchpole and Norton 1979; Coudert 1976, 1979; Licois and Coudert 1982; Licois et al. 1978a, b; Peeters et al. 1981). Although *E. intestinalis* is not the most common coccidium, Peeters et al. (1981) and Zundel et al. (1980) have found it in >21% of their battery-reared animals. Moreover, we have demonstrated that *E. intestinalis* has considerable immunogenicity (Licois and Coudert 1980a). Therefore, we felt that it would be of interest to obtain a precocious line of this species and characterize it.

**Materials and methods**

**Animals**

We used 6- to 7-week-old New Zealand rabbits (INRA strain A 1077), which were obtained free of coccidia from the Station de Pathologie Aviaire et de Parasitologie de l'INRA de TOURS and reared as specific pathogen-free (SPF) animals (Schellenberg 1976; Coudert et al. 1979; Coudert et al. 1988).

**E. intestinalis**

The method used to obtain the original strain (EiO), i.e. the parental strain, and the precocious line (EiP) is indicated in Table 1.

**Original strain.** Beginning with a mixture of *E. intestinalis* and *E. magna* isolated in 1975 from the caecal content of a rabbit with coccidiosis, we formed five lines in 1985 by inoculating five animals with one *E. intestinalis* oocyst each. Multiplication of the mixture of the five lines thus obtained in another animal provided the normal or original strain (EiO). In fact, for each multiplication, the animals were sacrificed 10 days after infestation, which corresponds to the excretion peak in this species. The strains were then isolated from the caecal content.
Table 1. Selection of EiO and EiP strains

<table>
<thead>
<tr>
<th>Date</th>
<th>Strain</th>
<th>Dose of oocysts given</th>
<th>First detection of oocysts in the caecum (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>Mixed strains of <em>E. intestinalis</em> and <em>E. magna</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1985</td>
<td>Isolation of 5 lines of <em>E. intestinalis</em></td>
<td>1 oocyst per line</td>
<td>8.5 (10)</td>
</tr>
<tr>
<td>24.01.1986</td>
<td>PA 1986.01 = parent strain (EiO)</td>
<td>4,000</td>
<td>8.5 (10)</td>
</tr>
<tr>
<td>9.12.1986</td>
<td>PA 1986.01/1</td>
<td>100,000</td>
<td>8</td>
</tr>
<tr>
<td>13.01.1987</td>
<td>PA 1986.01/2</td>
<td>&lt;10,000</td>
<td>7.5</td>
</tr>
<tr>
<td>3.02.1987</td>
<td>PA 1986.01/3</td>
<td>&lt;10,000</td>
<td>8</td>
</tr>
<tr>
<td>17.02.1987</td>
<td>PA 1986.01/4</td>
<td>&lt;10,000</td>
<td>7.5</td>
</tr>
<tr>
<td>3.06.1987</td>
<td>PA 1986.01/5</td>
<td>&lt;10,000</td>
<td>7.5</td>
</tr>
<tr>
<td>16.06.1987</td>
<td>PA 1986.01/6 = precocious strain (EiP)</td>
<td>10,000</td>
<td>5.5 (6)</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent the day of oocyst recovery for the preparation of EiO and EiP inocula.

*Precocious strain.* The EiP strain was derived from the original strain after only six passages. The oocysts were recovered in the caecum during the last multiplication, 6 days after infection.

*Excretion curve and rate of multiplication.*

Two separate rooms comprising 6 cages each contained 12 animals divided into 3 identical lots; there were 4 rabbits per lot and 2 animals per cage. In one room, the rabbits were inoculated with the EiP strain at doses of 50, 500 and 5000 sporulated oocysts corresponding to each lot. The rabbits in the other room were inoculated with the same doses of the EiO strain. The excreta were harvested every day between post-inoculation days 5 and 14. The coccidia counts were made according to a method previously described by Licois and Coudert (1980b).

*Morphological characteristics and sporulation time of the oocysts.*

These two criteria are among the most simple to use in poultry for the diagnosis of the different species of *Eimeria* (Norton and Chard 1983). Measurements of the sporulated oocysts were determined for both the EiP and EiO strains according to the technique described by Coudert et al. (1979) to check whether selection for precociousness does not change the size of the oocysts.

In the rabbit, detailed studies of sporogony were carried out by Coudert et al. (1973) in *E. stiedai* and by Coudert et al. (1979) in *E. perforans.* We carried out a similar study of the sporogony of the EiP and EiO strains and followed the change in this development at three different temperatures: 18.0°±0.5°C, 22.0°±0.5°C and 26.0°±0.5°C. To complete the morphological observation, the evolution of the number of completely sporulated oocysts (infective oocysts) as a function of time was determined by inoculating the rabbits at 4-h intervals between 49 and 73 h with aliquot samples of the oocyst suspension that was allowed to sporulate at 22° C. The control inoculum corresponding to 100% sporulation was obtained at 85 h.

The inocula were prepared by taking the assumed percentage of sporulation into account according to the morphology so as to inoculate 5,000 sporulated EiP oocysts per animal and 500 of the EiO strain. These doses were chosen since, especially for the EiO strain, parasite excretion increases in proportion to the inoculum, but only within a range of 1-1,000 inoculated oocysts.

*Pathogenicity.*

Two experiments were carried out to determine pathogenicity. The first experiment compared the EiP strain with the EiO strain. Eight rabbits per dose were infected with 50, 500, 5,000 and 50,000 oocysts of each strain. Eight non-inoculated animals were used as controls. Pathogenicity was determined by measuring weight gain in the animals, with individual weighings carried out twice a week.

In the second experiment, we compared the evolution of weight gain of rabbits inoculated with doses of 4.6×10³, 15.5×10³, 9.3×10³, 8.8×10³ and 2.4×10³ EiP oocysts. There were 18 animals/dose and a control group of 18 non-inoculated rabbits. The animals were individually weighed twice a week. Moreover, oocyst excretion was evaluated over two periods ranging from days 4 to 10 and days 10 to 14 after inoculation.

*Results.*

*Excretion curve and rate of multiplication.*

Figure 1 provides the results for the daily output of oocysts from the EiP line as compared with...