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EIMERIA MEDIA: SELECTION AND CHARACTERIZATION OF A PRECOCIOUS LINE

D. LICOIS, P. COUDERT, F. DROUET-VIARD, M. BOIVIN

I.N.R.A. Laboratoire De Pathologie du lapin. Station de Pathologie et de Parasitologie. 37380 MONNAIE. FRANCE.

INTRODUCTION

Up to date, only inoculation with live coccidia, if possible with attenuated pathogenicity, provides sufficient protection against coccidiosis (Long and Rose, 1982; Rose, 1982, 1986; Rose and Long, 1980). In this perspective, precocious lines of coccidia obtained by selection for early development of oocysts have been extensively studied in poultry (Jeffers, 1975; Johnson et al., 1986; Mc Donald and Ballingall, 1983a, b; Mc Donald et al., 1982, 1986; Shirley et al., 1984, 1986; Shirley and Bellatti, 1984).

In the rabbit, such a line has been obtained and characterized for *Eimeria intestinalis* (Licois et al., 1990). In the present study, a precocious line of *E. media* was developped. This species is not very pathogenic (Licois and Coudert, 1982; Coudert et al., 1988a) but is widespread in rabbits husbandry (Zundel et al., 1980). The morphology, reproduction, pathogenicity and immunogenicity of this precocious line were compared with those of the parent strain.

MATERIAL AND METHODS

- Experimental animals

6 to 7-week-old, coccidia free, New Zealand White rabbits (INRA A1077) were used. They were reared like SPF animals (Coudert et al., 1988b). They were randomized, two per cage, at weaning (31 $\pm$ 1 days), according to their weight at weaning and their original litter. Experimental rooms and materials were desinfected by steam at 120°C followed by formol vapor. The other experimental conditions were previously described (Coudert et al.,1979).

- *E. media*

The original (parent) strain (OEmed) was a mixture of five wild strains of *E. media* isolated in France, Guadeloupe, Balearic Isles, Ivory Coast and Poland, and subsequently purified by multiplicating single oocysts. The precocious line (PEmed) was developped from the mixture by repeated selection of the first excreted oocysts and serial passages in animals (Table 1). Freshly sporulated oocysts, recovered on day 6 for the parent strain and on day 3 for the precocious line, after multiplication, were used as inoculum in the tests for pathogenicity and immunogenicity.

- Experimental designs

Two experiments were carried out.

In the first one, seven groups of 8 animals were used. One group, used as control, was not inoculated. Five groups were given, as a primary inoculation, 1.10^3, 1.10^4, 1.10^5, 1.10^6 and 1.10^7 oocysts of the PEmed line. The last group received 7.10^4 oocysts of the OEmed strain. All the rabbits of the infected groups were challenged, thirteen days later, with 7.10^4 oocysts of the parent strain. In addition, for this second inoculation, two other age-matched groups of 8 rabbits were used. One of them was not inoculated and the second one was infected (first inoculation) with 7.10^4 oocysts of the OEmed strain. Animals were weighed 2 times a week. After the first inoculation, daily oocyst output was monitored between days 4 and 7 for all the rabbits of each group. After the challenge total oocyst output was determined in all the rabbits of each group.

In the second experiment, five groups of 14 rabbits were used. One group (control) was not given oocysts. Three groups were infected for the first time with 1.10^3, 1.10^5 and 1.10^7 PEmed oocysts whilst the fourth group was given 7.10^4 OEmed oocysts. Thirteen days later, 10 animals of each inoculated groups were challenged with 7.10^4 OEmed strain whilst the last 4 animals of the same inoculated groups, were reinfected with 1.10^5 oocysts of the precocious line. Animals were weighed 3 times a week. Daily oocyst output was monitored , in 8 rabbits of each group, between days 2 and 9 after the first infection. After the challenge, total oocyst output was determined in 8 rabbits of each group reinoculated with the OEmed strain and in the 4 rabbits of the group reinoculated with the PEmed line.

RESULTS

- Development of the precocious line and morphology. The prepatent period of *E. media* has been given as 120 hr (Cheissin, 1972; Licois and Coudert, 1982; Coudert et al. 1988a). The table 1 shows that the prepatent time regularly decrease to 90 hr during the 4 first passages. From the 5th to the 9th generation selection had to be relaxed to increase the yield of oocysts. Then to the 10th to the 12th passage another reduction of the prepatent time was performed and by the 12th generation oocysts could be recovered at 72 hr.

In the original strain, the oocysts contained four identical sporocysts, each of them with two sporozoites and a residual sporocyst body. Each of the sporozoites enclosed a refringent globule. So there were 8 refringent globules per oocyst.

In the precocious line, all the oocysts were similar with four identical sporocysts but different from those of the wild strain. Each sporocyst contained a large refringent globule about the size double of that of the parent strain. Then these oocysts harbored 4 huge refringent globules. Microscopic observation of sporulated oocysts showed that the morphological changes occured at least from the 5th passage onwards with the presence in a few oocysts of 2 kinds of sporocysts. One of them resembled to that of the parent strain and the other one was like that of the precocious line. Nevertheless, the majority of oocysts from the 5th passage and all the oocysts coming from the following passages were those including sporocysts of the precocious line.

- Multiplication rate and excretion curve. The first oocysts were detected 60 hr after the inoculation in rabbits infected with the PEmed line instead of 96 hr in those given the OEmed strain (Fig. 1a). The peak of excretion was on day 5 (or 6) for the PEmed line (Fig 1a, 1b) and on day 7 for the parental one (Fig 1b).

In both experiments, the total oocyst production increased with the dose (Fig 1b). The maximum of production was obtained with at least 1.10^5 for the PEmed line (Table 2). In the case of the parental strain, the maximum was already reached with 7.10^4 oocysts inoculated. So for both strains the same level ($2\text{-}4.10^8$ oocysts) was produced. In fact one can know that with a wild strain of *E.media* the maximum of oocyst shedding is obtained with about a hundred of oocysts inoculated.

- Pathogenicity.

Symptoms: Similar results could be seen for both experiments. No mortality was observed in any group. Some cases of diarrhea were noticed in animals inoculated with the OEmed strain or with the higher doses of the precocious line, but this diarrhea was mild and of short duration.

Weight gain: (Fig 2a, 2b): For both experiments, in control groups, the mean daily weigh gain was about 40 g. No difference was seen between these control groups and the rabbits given 1.10^3 oocysts of the PEmed line.

In animals inoculated with 1.10^4, 1.10^5 or 1.10^6, a depression of the weigh gain which increased with the dose could be noticed. It occured during the 3 first days after the infection up to day 6 or 7, while in rabbits inoculated with the OEmed strain, the depression of the weigh gain was marked later, between the 3rd and the 6th day. The figure 2b shows that it was necessary to inoculate at least 1.10^7 PEmed oocysts to obtained the same depression of the weight gain that that induced by 7.10^4 OEmed oocysts. From 6-7 days postinoculation, the weight gain of inoculated animals was comparable to that of the control groups.

- Immunogenicity.

Weight gain: In the first experiment, the evolution of the weight gain of the rabbits immunized with the OEmed strain was similar to that of the control group (Fig. 3a). All the rabbits inoculated as a primary infection with the PEmed line had a depression of their weight gain which occured between days 0 and 4 after the challenge. The figure 3a shows that this depression of the weight gain was almost the same for the rabbits first inoculated with 1.10^3, 1.10^4 or 1.10^5 than that observed in non-immunized rabbits; the protection was better in rabbits immunized with 1.10^6 and 1.10^7 PEmed oocysts. After, whereas the weight gain of the non-immunized animals remained low, that of the rabbits immunized with the precocious line had a strong increase of their weight gain, marked between days 4 and 8 after the challenge.

In the second experiment, one can also observe that the weight gain of the non-immunized rabbits strongly decreased from days 3 to 6 after the challenge, before to become again similar to that of the control group (Fig. 3b). No significative difference was noticed between the weight gain of the rabbits immunized with the precocious line and that of the control group. The figure 3c shows taht rabbits given the PEmed line were completely immunized towards a challenge with 1.10^5 oocysts of the same precocious line.

Oocyst output: (Table 2) For both experiments, the total oocyst output in non-immunized rabbits reached its maximum at the level of 1 - 3.10^8. In animals first inoculated with the OEmed strain and reinoculated with the same inoculum, a good but not total protection could be noticed as 2 to 8.10^5 oocysts were excreted. In rabbits inoculated with the PEmed line and challenged with the OEmed strain the oocyst shedding was inversely proportional to the dose used at the primary infection. In rabbits initilly inoculated with the PEmed line and challenged with 1.10^5 oocysts of the same PEmed line the protection was nearly absolute since the the total oocyst output was equal or less that 5.10^4 which is the threshold of the counting.

DISCUSSION

Repeated Selection for precocious development of oocysts has made it possible to obtain a precocious line of *E. media*. Within 7 generations, if we except those necessary to enlarge the inoculum, the prepatent period has been reduced by 40% (from 120 to 72 hr). The study of the excretion curve shows that the first oocysts could be detected at 96 hr for the OEmed strain and at 60 hr for the PEmed line. One explanation of this fact is propably due to the sensitiveness of the method of counting used.

Surprisingly, as for *E. intestinalis* (Licois et al., 1990), morphological changes were seen in the sporocysts of the PEmed line but with a difference. In the precocious line of *E. intestinalis* 2 of 4 sporocysts contain a very large refringent globule whereas in the PEmed line each sporocysts enclosed also a huge refringent globule. .The same last observation can be done for another precocious line of *E. magna* we have also obtained. For all these species, only few generations (five in the case of *E. media*) were necessary to obtain oocysts with morphological anomalies This constant reduction of number of refractile bodies and the increase of their size associated with the appearace of a shortened life cycle suggest some further studies on the genetic of Eimeria.

Compared to the parental strain, the precocious line of *E. media* appeared less pathogenic and it was necessary to inoculate at least 1.10^7 PEmed oocysts to have the same results than 7.10^4 oocysts of the OEmed strain, as regards the weight gain. The ability of the rabbit to multiply the PEmed line was not altered, since the same maximum level of oocysts produced was reached for the both strains. However, the reproductive potential of each oocyst of the PEmed line was much lower (about 1000 times). A similar decease of the multiplication rate of precocious lines was also noticed with *E. intestinalis* (Licois et al., 1990) and *E. magna*. Concerning the immunogenicity, the PEmed line seemed rather efficient as regards the weight gain but did not prevent totally the oocyst output, except against itself. These characteristics of the PEmed line are in agreement with those reported for the precocious lines in poultry (Jeffers, 1975; Johnson et al., 1986; McDonald and Ballingall, 1983a, b; McDonald et al., 1982, 1986; Shirley and Bellatti 1984; Shirley et al.,1984, 1986). Only Bedrnick et al. (1986) report that their precocious line of *E. tenella* does not lose its multiplication rate and virulence. Preliminary results seems to indicate that the PEmed line is stable after further passages in animals. To try to explain the differences observed between the parent and the precocious strains investigations on the endogenous development are in progress.

In view of these data the precocious line of *E. media* appears to be suitable for a use as a live attenuated vaccine.

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ABSTRACT

A precocious line of *Eimeria media* was obtained by selection for early development of oocysts in rabbits. The prepatent period was reduced from 90 to 60 hr. The precocious line was less pathogenic than the parent strain and its multiplication rate was lower. Rabbits given oocysts of the precocious line were immunized to challenge with the wild strain as regards the weight gain, a criteria of pathogenicity, although they were not totally protected towards the oocyst output. Selection for precocious development was accompanied by morphological changes in the sporulated oocysts; each sporocyst contained a large refractile body instead of two smaller in the parent strain.

FIG 1a: Mean daily oocyst output of the PEmed line
Experiment 1

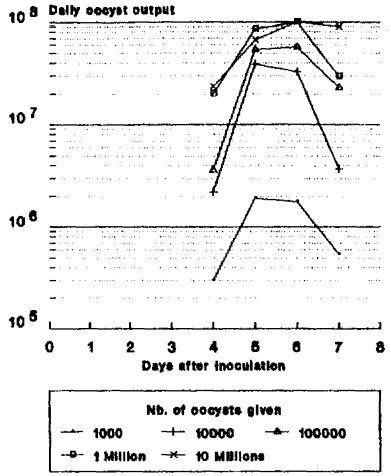


FIG 1b: Mean daily oocyst output of the OEmed strain and the PEmed line
Experiment 2

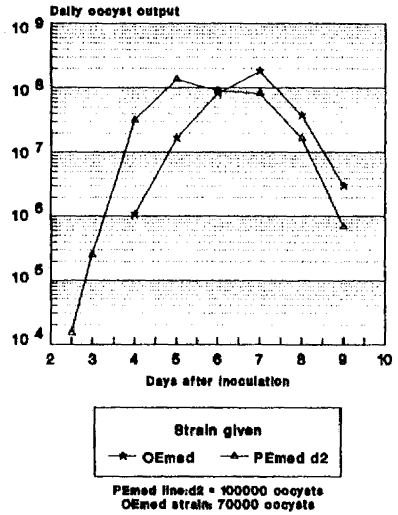


FIG 2a: Mean daily weight gain of rabbits inoculated with different doses of the PEmed line
Experiment 1

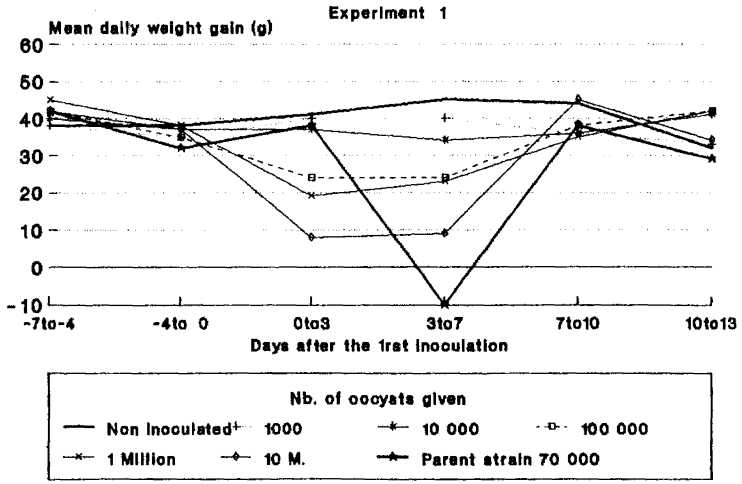
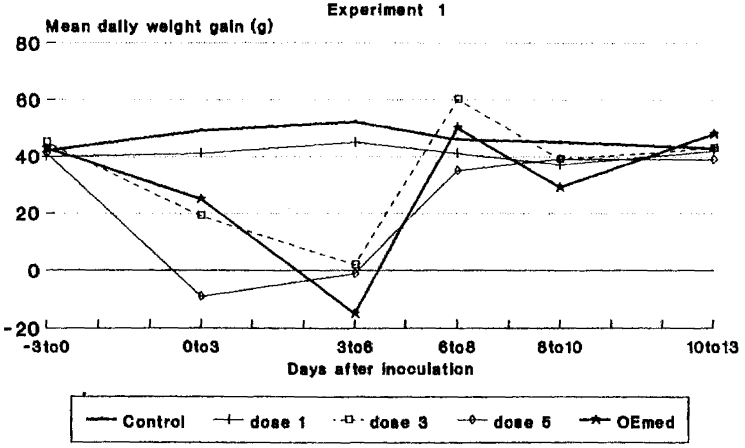
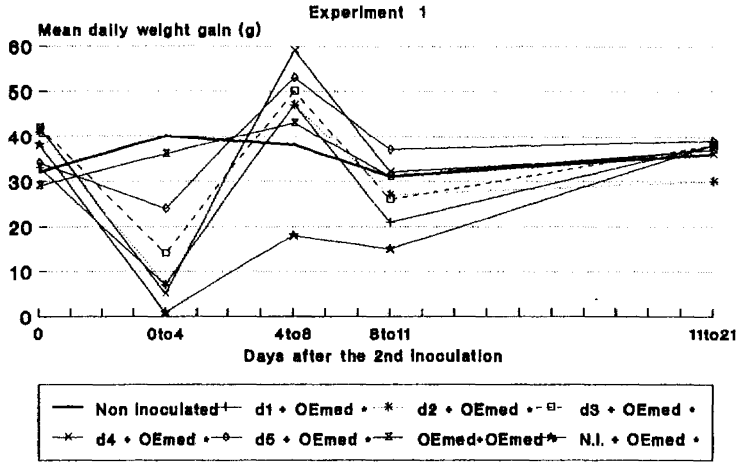


FIG 2b: Mean daily weight gain of rabbits inoculated with different doses of the PEmed line
Experiment 1



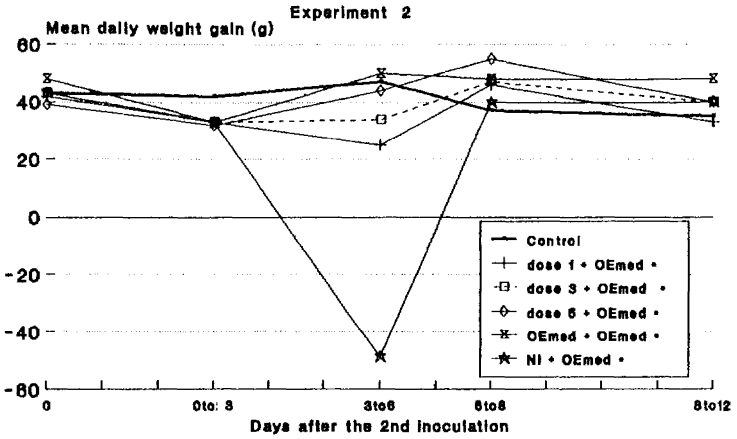
doses 1, 3 & 5 : PEmed line 10³ 10⁵ & 10⁷
OEmed: Parent strain 7 x 10⁴
Control: non inoculated animals

FIG 3a: Immunogenicity of the PEmed line
Mean daily weight gain after a challenge
with 70000 OEmed oocysts



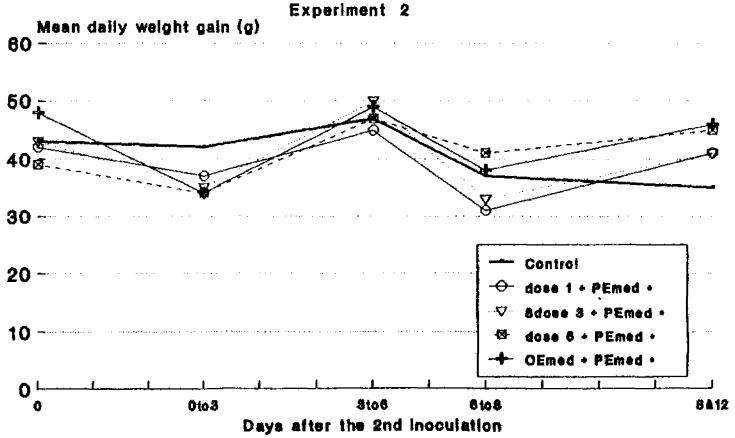
d1=10 3 d2=10 4 d3=10 5 d4=10 6 d5=10 7 PEmed line (1rst inoc)
OEmed: Parent strain 70000 oocysts

FIG 3b: Immunogenicity of the PEmed line
Mean daily weight gain after a challenge
with 70000 OEmed oocysts



doses 1, 3 & 5 : PEmed line (1rst inoc) 10 3 10 5 & 10 7 oocysts
OEmed strain 70000 oocysts. • 2nd inoc 7 x 10 4 oocysts
Control : non inoculated animals N.I. Non inoculated at the 1rst inoculation

FIG 3c: Immunogenicity of the PEmed line
Mean daily weight gain after a challenge
with 100000 PEmed oocysts



doses 1, 3 & 5: PEmed line (1rst inoc) 10 3 10 5 & 10 7 oocysts
OEmed: Parent strain 7 x 10 4 oocysts
Control: non inoculated. • 2nd inoc Inoculation

Table 1. Selection of the precocious line of *Eimeria media*.

Passage no.	Strain	Number of oocysts given	Time after inoculation when 1rst oocysts were excreted (hours)
	Parent strain = OEmed (mixture of 5 wild strains of *E. media*)		108
1	PA 1991-20	ND	98
2	PA 1991-21	$5.2x10^4$	93
3	PA 1991-22	$4\ x10^3$	92
4	PA 1991-23	$1.4x10^5$	90
5	PA 1991-24	ND	90 *
6	PA 1991-25	$2.5x10^5$	90
7	PA 1991-26	$2.5x10^5$	90
8	PA 1991-27	$2.5x10^5$	90
9	PA 1991-28	$2.5x10^5$	90
10	PA 1991-29	$2.5x10^5$	86
11	PA 1991-30	$2.5x10^5$	78
12	PA 1991-31 =PEmed	$2.5x10^5$	72

ND Not detectable
* 1rst appearance of sporocysts including a huge refractile body typical of the precocious lines from Eimeria of the rabbit.

Table 2. Multiplication rate (Experiment 1 and 2)
Total oocyst output, after the 1rst inoculation, in rabbits inoculated either with 7.10^4 OEmed oocysts or with different doses of PEmed oocysts and total oocyst output after a challenge with either 7.10^4 OEmed or 1.10^5 PEmed oocysts (x 1.10^6).

	1rst inoculation		2nd inoculation	
	Strain and dose of oocysts given	Total oocyst output Day 4 to day 7	Strain and dose of oocysts given	Total oocyst output Day 3 to day 9
1rst Experiment	Non-inoc.	0 *	7.10^4 OEmed	295
	7.10^4 OEmed	310	" "	0.8
	1.10^3 PEmed	5	" "	15
	1.10^4 "	77	" "	27.4
	1.10^5 "	137	" "	13.1
	1.10^6 "	236	" "	4.6
	1.10^7 "	292	" "	3.6
2nd Experiment	Non-inoc	0 *	7.10^4 OEmed	94.5
	7.10^4 OEmed	357	" "	0.2
	1.10^3 PEmed	14	" "	5.8
	1.10^5 "	354	" "	7.4
	1.10^6 "		" "	3.5
			1.10^5 PEmed	0.05
			" "	0 *
			" "	0 *
			" "	0 *

* The threshold of detection of oocysts with the method used is 5.10^4
All the numerations are the mean of 8 rabbits except for the animals challenged with the PEmed line in the 2nd experiment where 4 rabbits were used.