ACQUIRED PROTECTION AGAINST *EIMERIA MAGNA* COCCIDIOSIS USING A PRECOCIOUS LINE : EFFECT OF VACCINE DOSE AND AGE AT VACCINATION

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

INRA, Laboratoire de Pathologie du Lapin, Station de Pathologie aviaire et Parasitologie, F-37380 Nouzilly.

Abstract - Twenty-five, twenty-seven- and twenty-nine-day-old sucklings were vaccinated orally with a precocious line of *Eimeria magna*. Each group received two doses varying from 3.5 x 10^2 to 3.5 x 10^4 oocysts. At 36 days of age, animals received a challenge inoculation with 10^4 oocystes of the wild strain of *E.magna*. Vaccination reduced oocyst output 10 to 1000 times after the challenge inoculation and prevented the decrease in the weight gain observed in non vaccinated challenged animals. When the vaccination was performed more than 9 days before challenge, the protection can be total An individual oral vaccination performed with 3500 oocysts gave total protection whatever the age at vaccination between 25 and 29 days of age.

INTRODUCTION

E.magna is one of the most common coccidia found in intensive rabbit husbandries (PEETERS *et al.* 1983). It causes important economical losses: diminution of weight gain of growing rabbits and sometimes cases of diarrhea and mortality. This affection occurs mainly in weanling rabbits (5 to 6 weeks old) which are not protected by the immunity acquired by their mother (DROUET-VIARD *et al.* 1994a). So, the desease must be prevented very early. LICOIS *et al* (1995) already tested the immunogenic effect of an *Eimeria magna* precocious line. Previous works (DROUET-VIARD *et al.* 1994b) showed that rabbits could be incompletely protected after a vaccination at 25 days of age with 3.5 x 10^4 oocysts of a living precocious line, and that a vaccination with the same dose at 29 days of age gave total protection. The purpose of this work was to test the effect of an intermediate age (27 days) of the sucklings at vaccination to improve the protection acquired after a vaccination at 25 days of age. We tested three vaccine doses to determine more accurately the relationship with the degree of acquired protection.

MATERIAL AND METHODS

Animals

The young rabbits were the offspring from 18 litters of New Zealand white females reared in standard conditions since their first gestation; they were at their third kindling. The controls came from our coccidia free rabbitry (COUDERT *et al.*, 1988),. All the does were fed a Robenidine supplemented commercial pelleted feed; the supplemented feed was replaced by non-supplemented pelleted feed four days before vaccination, (UAR 91360 Villemoison/Orge, France). Housing conditions were those described by DROUET-VIARD *et al.* (1983).

Parasites

The oocysts used for the vaccination and for the challenge inoculation were recently sporulated and kept at 4°C in a 2.5% potassium bichromate water solution. We vaccinated the animals with the precocious line *E.magna* 1992-29 developed from the reference line PrEmag 1990-12. Challenge inoculation was performed with a wild strain, *E.magna* 1993-38, derived from the reference strain OrEmag 1988-01; the precocious line was also obtained from this reference strain.

The method used for counting the number of oocysts excreted is that of COUDERT *et al.* (1995). Countings were performed per cage and the results expressed per animal.

Experimental design (Table 1)

Sucklings were vaccinated orally once at 25, 27 or 29 days of age with the precocious line. Three doses were tested: d1 = 3.5×10^2, d2 = 3.5×10^3 and d3 = 3.5×10^4 oocysts. For each age of vaccination, 5 litters were used. In each litter the sucklings were randomly vaccinated with two of the vaccine doses. Coccidia-free weanlings of the same age were used as control animals.
All the young were weaned when 30 days old and put into cages of 3 animals. At 36 days of age, animals received a challenge inoculation with 10^4 oocystes of the wild strain of *E.magna*. The inoculation was performed on the same day for all groups, i.e. 11, 9 or 7 days after vaccination. Part of the vaccinated rabbits of each group were not challenged to assess a possible long-term disease due to vaccination.
3 doses of oocysts of a precocious line of *E. magna* were tested for oral vaccination.
The whole oocyst output was measured in each group between day 5 and day 9 after vaccination and between day 4 and day 10 after challenge inoculation; for both strains, during these intervals, more than 90% of the oocysts are excreted. Animal weights and fecal consistency were regularly recorded.

Table 1: Repartition of animals in the experimental groups

	Age at vaccination			Challenge
vaccine dose	25 days	27 days	29 days	35 days
-	-	-	-	+ no vac chal
d1 = 3.5×10^2	/	/	V29d1	+
d2 = 3.5×10^3	V25d2	V27d2	V29d2	+
d3 = 3.5×10^4	V25d3	V27d3	/	+
-	-	-	-	- controls

Statistical analyses

Tukey test (HSD multiple comparisons program SYSTAT) was used to compare the means of weight gains.

RESULTS

1/ Oocyst output after vaccination and after challenge inoculation (Table 2)

We checked the excretion of the vaccine line 9 days after vaccination. All the animals multiplied the vaccine line (excretion over 6.5×10^6 oocysts).
After the challenge inoculation, a decrease in the output of oocysts was recorded in all the vaccinated groups. In rabbits vaccinated at 29 days i.e. 7 days before challenge, there was a non neglectable oocysts output and probably a dose effect

Table 2 : Oocysts excretion after the vaccine inoculation and after challenge

group	age at vaccination	dose	vaccine excretion	challenge excretion
V25d2	25	d2	$6,7 \times 10^6$	ud
V25d3		d3	$9,3 \times 10^6$	ud
V27d2	27	d2	$2,8 \times 10^7$	$9,1 \times 10^5$
V27d3		d3	$6,6 \times 10^7$	ud
V29d1	29	d1	$1,2 \times 10^7$	$2,6 \times 10^7$
V29d2		d2	$1,7 \times 10^7$	$6,5 \times 10^6$
no vacc chal	non-vaccinated	/	ud	$1,1 \times 10^8$
unchallenged controls	non-vaccinated	/	ud	ud

2/ Weight gain patterns (Figure 1)

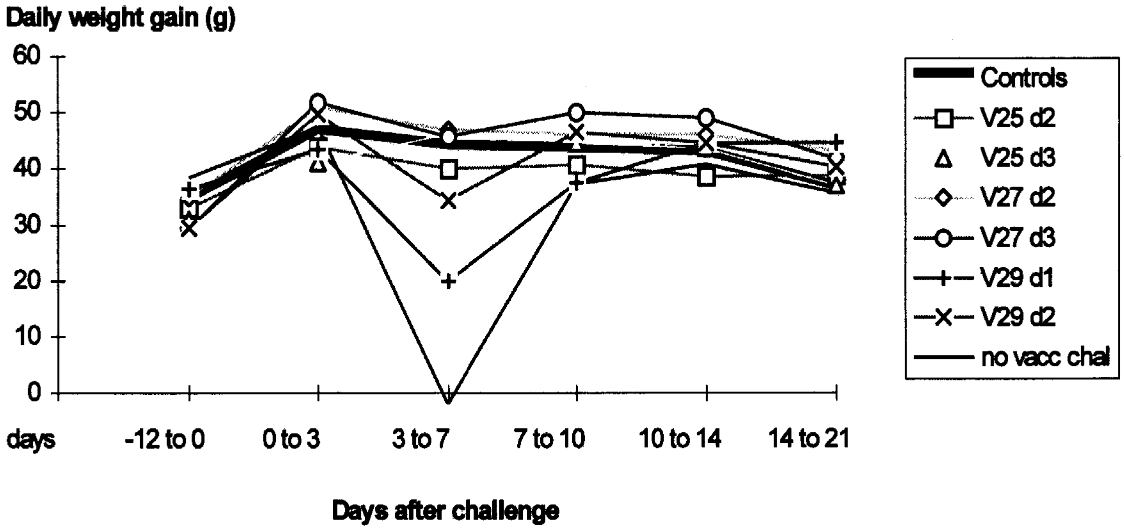
The only significant differences observed in the weight gain pattern between the experimental groups were registered between day 3 and day 7 after challenge inoculation ($P<0.001$):

- - non-vaccinated challenged animals lost weight (weight gain significantly different from all the other groups, $P<0.001$);
- - no difference was observed in the weight gain pattern between unchallenged controls and groups vaccinated at 25 or 27 days of age.
- - a decrease in weight gain was observed in animals vaccinated at 29 days of age, varying according to the vaccine dose given. The weight gain observed in rabbits vaccinated with d2 at 29 days of age is not significantly different ($P> 0,1$) from the weight gain of rabbits vaccinated earlier, though the weight gain of this group was rather lower than in other groups. In the group vaccinated with the lowest dose (d1) the weight gain was significantly lower ($P<0.001$) when compared to the other vaccinated groups and was significantly higher ($P<0.001$) when compared to that of the non-vaccinated challenged animals.

The young rabbits were vaccinated orally at the ages of 25, 27 or 29 days with an oocysts dose of the precocious line of *Eimeria magna*, d1 = 3.5×10^2, d2 = 3.5×10^3 and d3 = 3.5×10^4 oocysts. They were given a challenge inoculation with 10^4 oocysts of a wild strain of *E.magna* at 36 days of age. The threshold of detection for the method used is 10^2 oocysts/g of feces; in several groups excretion was undetectable (ud).

The sucklings were vaccinated per os individually with either dose d2 = 3.5×10^3 or d3 = 3.5×10^4 oocysts of *E.magna* precocious line at 25 or 27 days of age (V25d2, V25d3, V27d1, V27d3), and with d1 = 3.5×10^2 or d2 = 3.5×10^3 at 29 days of age (V29d1, V29d2). A challenge inoculation per os with 10^4 oocysts of a wild strain of *E.magna* was performed at 35 days of age. Unvaccinated animals were either challenged (no vac chal)or unchallenged and used as Controls.

Figure 1: Weight gain patterns invaccinated animals after a challenge inoculation



DISCUSSION - CONCLUSION

The precocious line of *Eimeria magna* was able to protect weanlings against a wild strain challenge inoculation, whatever the age at vaccination, 25, 27 or 29 days of age. Nevertheless, the effect depended on the intervals between vaccination and challenge, and to a lesser level on the vaccine dose.
In this trial, a dose of 35000 oocysts gave total protection (undetectable oocyst output and no diminution of weight gain when vaccination was performed at 25 or 27 days of age. This result ascertains the results of DROUET-VIARD *et al.* (1994)
With a dose of 3500 oocysts total protection was acquired by the sucklings vaccinated at 25, 27 or 29 days of age. These results complete our previous results (DROUET-VIARD *et al.*, 1994): with this dose in 25-day-old animals challenged 9 days after vaccination, the animals were not completely protected, but in this trial with a challenge performed 11 days after vaccination there was total protection. So, it is highly probable that in our first experiment (DROUET-VIARD *et al.*, 1994), the dose was sufficient but the time allowed for acquisition of complete immunity was not long enough.

We demonstrated in this trial that a very low vaccine dose of 350 oocysts can protect partially the animals when given more than 7 days before challenge; this result is in agreement with the results obtained when using spray dispersion of the vaccine in the nest-box (DROUET-VIARD *et al.*, 1996).
We can conclude that vaccination is efficient when performed as early as 25 days which gives time to the sucklings to develop sufficient immunity at the time of weaning. It is also important to point out that this experiment indicates that complete immunity can be acquired very quickly (9 days) after the first contact with the parasite.
A vaccination could be performed successfully at 27 days of age; its interest would be the possibility of using a lower vaccine dose.

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Essai de vaccination contre une coccidiose à *Eimeria magna* à l'aide d'une lignée précoce - Des lapereaux non sevrés âgés de 25, 27 ou 29 jours ont été vaccinés per os avec une lignée précoce d'*Eimeria magna*. Chaque groupe recevait deux doses variant de 3.5×10^2 à $3,5 \times 10^4$ oocystes. A l'âge de 36 jours, les animaux ont reçu une inoculation d'épreuve avec 10^4oocystes d'une souche sauvage d'*E.magna*. La vaccination réduisait d'un facteur 10 à 1000 l'excrétion d'oocystes après l'inoculation d'épreuve, et supprimait la perte de gain de poids observée après l'inoculation d'épreuve chez les animaux témoins non vaccinés. Lorsque la vaccination était pratiquée plus de 9 jours avant l'inoculation d'épreuve, la protection des lapereaux pouvait être complète. Une vaccination individuelle par voie orale réalisée avec 3500 oocystes est totalement efficace quelque soit l'âge des lapereaux à la vaccination, entre 25 et 29 jours.