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VACCINATON TRIAL AGAINST *EIMERIA MAGNA* COCCIDIOSIS USING A PRECOCIOUS LINE.

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SUMMARY : Thirty six 25-day-old suckling rabbits and thirty six 29-day-old rabbits weaned since one day were orally vaccinated with one of the two doses (3.5×10^3 or 3.5×10^4 oocysts) of a precocious line of *Eimeria magna*. Only the groups vaccinated with the highest dose displayed a vaccine reaction, but without diarrhea or mortality. A vaccination totaly or incompletely protected against disease, assessed by daily growyh rate patterns, according to the dose given and reduced oocyst output 10 to 1000 times, measured between the 4th and the 10th day following the challenge inoculation.

RESUME: Essai de vaccination contre une coccidiose à Eimeria magna à l'aide d'une lignée précoce.
Trente six lapereaux non sevrés âgés de 25 jours et 36 lapereaux de 29 jours sevrés depuis une journée ont été vaccinés per os avec une des deux doses ($3,5 \times 10^3$ ou $3,5 \times 10^4$ oocystes) d'une lignée précoce d'Eimeria magna. Seuls les lots vaccinés avec la dose la plus forte ont eu une réaction vaccinale, n'entraînant cependant ni diarrhée ni mortalité. Le suivi de l'évolution de la vitesse de croissance a permis de constater que selon la dose administrée, la vaccination a protégé partiellement voire totalement contre la maladie et a réduit d'un facteur 10 à 1000 l'excrétion d'oocystes mesurée entre le 4ème et le 10ème jour suivant l'inoculation d'épreuve.

INTRODUCTION

E. magna is one of the most frequently found coccidia in intensive rabbit breeding units (PEETERS *et al.*, 1983). It causes considerable economic losses due to decrease in weight gain, diarrhea and even mortality. The disease mostly affects young rabbits just after weaning (5-6-week-old animals), and prevention of this disease must therefore be initiated as early as possible. LICOIS *et al.* (1991) demonstrated the immunogenicity of a precocious line of *Eimeria magna* in young weaned rabbits. Suckling rabbits in standard breeding conditions are not susceptible to infection before the age of 20 days (ROSE, 1959; COUDERT *et al.* 1991.).

An efficacious vaccination should therefore be performed between 20 and 30 days of age. In this trial, we compared the efficacy of oral vaccinations performed at 25 and 29 days of age (i.e. 4 days before or 1 day after weaning) and we tested two vaccine doses.

MATERIAL AND METHODS

Animals.

The young rabbits were the offspring from 16 litters of New Zealand white females from our coccidia free rabbitry (COUDERT *et al.* 1988): eight does had been previously immunised against *E. magna* with the precocious line (*E.magna* 1992-29), and the eight other does were naïve. As no effect of mother's immunisation on susceptibility of weanling rabbits to coccidiosis had been demonstrated (DROUET-VIARD *et al.* 1996) mothers of both status could be used for this experiment. All the does were fed a Robenidine supplemented pelleted feed (UAR 91360 Villemoison/Orge, France). Housing conditions were those described by VIARD-DROUET *et al.* (1983). The supplemented feed was replaced by non-supplemented commercial pelleted feed four days before vaccination, (UAR 91360 Villemoison/Orge, France).

Parasites.

The oocysts used for the vaccination and for the challenge inoculation were recently sporulated (less than 2 months) 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* or 1993-38, derived from the reference strain OrEmag 1988-01; the precocious line derived from this reference strain by selecting the first oocysts excreted as described by LICOIS *et al.*(1995). 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.

Oocysts used for vaccination and challenge purpose were administered per os.

1/Vaccination.

Eight litters (4 from immunised does and 4 from naïve does) were vaccinated at 25 days of age; half of each litter was vaccinated with 3.5×10^3 oocysts of the precocious line of *E. magna,* the other half with 3.5×10^4 oocysts .

Eight other litters (4 from immunised does and 4 from naïve does) were vaccinated at 29 days of age in the same conditions.

Nine coccidia-free young rabbits born from 3 does were used as control animals (non-vaccinated challenged).

2/ Challenge inoculation

All the young were weaned when 28 days old (more than 81 rabbits) and divided to 9 groups. For each

group, 9 rabbits were randomised into 3 cages of 3 animals (Table 1). All animals received a challenge inoculation with 10^4 oocysts of the wild strain of *E.magna*. The inoculation was performed on the same day for all groups when rabbits were 34 days old, i.e. 9 or 5 days after 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 were regularly recorded.

Statistical analyses

NEWMAN-KEULS test (variance analysis program STATITCF) was used to compare the means of weight gains.

RESULTS

None of the parameters recorded were affected by the immune status of the does.

1/ Oocyst output after vaccination and after challenge inoculation.

We checked the excretion of the vaccinal strain by the vaccinated animals 9 days after vaccination (Table 1). All the animals multiplied the parasite (excretion over 6.5×10^6 oocysts).

The excretion of oocysts of the wild strain was measured between day 4 and day 10 after the challenge inoculation (Table 1). A decrease in the output of oocysts was recorded in all the vaccinated groups, the best result being obtained with vaccination at 25 days of age with 3.5×10^4 oocysts.

2/ Weight gain patterns

a) Weight gain after vaccination (figures 1 and 2).

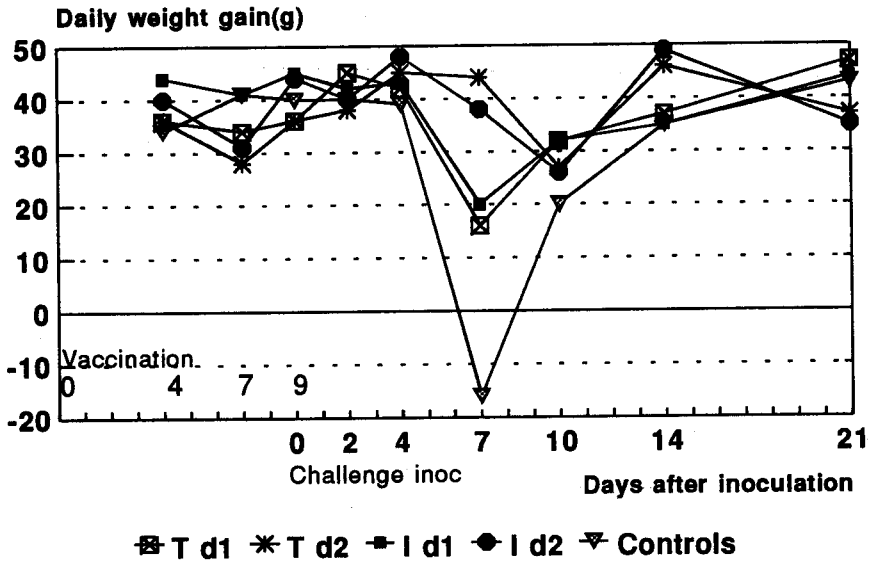
Vaccination at 25 days of age did not induce a variation in weight gain. On the other hand, vaccination at 29 days of age caused a sharp decrease in weight gain ($P < 0.1\%$); this difference in the vaccine reaction is probably related to the sharp increase in the sensitivity to coccidial infection of rabbits between 23 and 30 days of age.

Table 1 : Oocyst output after vaccination and challenge inoculation.

Vaccination		Mother's status	Excretion (oocysts/g)	
Age (days)	Doses of oocysts		After vaccination	After challenge innoculation
25	3.5×10^3	Naïve	6.5×10^6	5.9×10^7
		Immunised	not measured	2.4×10^7
	3.5×10^4	Naïve	not measured	1.2×10^5
		Immunised	2.3×10^7	3.4×10^5
29	3.5×10^3	Naïve	6.7×10^7	5.9×10^6
		Immunised	not measured	2.9×10^6
	3.5×10^4	Naïve	not measured	7.4×10^6
		Immunised	7.3×10^7	2.5×10^6
Non vaccinated controls			-	1.7×10^8

The young rabbits (9 per group) were the offspring of naïve or immunised females. They were given a challenge inoculation with 10^4 oocysts of a wild strain of *E.magna* when rabbits were 34 days old. The oocyst output values correspond to the average excretion by one animal; each value is the mean value of the excretions of the three cages of the group and for each cage the value corresponds to the whole excretion of the cage divided by 3.

Figure 1 : Weight gain of young rabbits vaccinated at 25 days of age with the precocious line of *Eimeria magna*



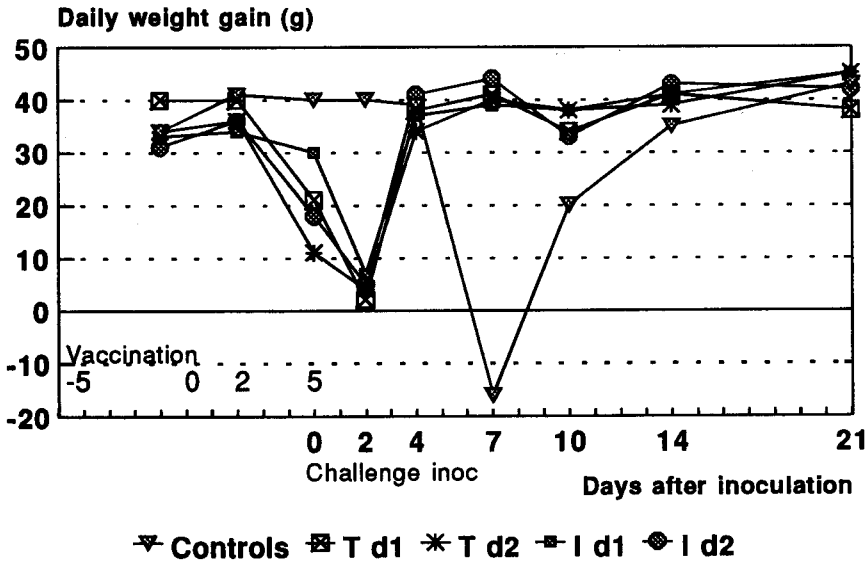
T: rabbits born of naive does ; I : rabbits born of immunised vaccinated does
Vaccine doses : d1 = 3.5 x 10^3 oocysts or d2 = 3.5 x 10^4 oocysts.

b) Weight gain after challenge inoculation (Figures 1 and 2).

The weight gain pattern after challenge inoculation was significatively different according to the experimental group (P<0.1%):

- non-vaccinated control animals lost weight, the minimum being observed 7 days after the challenge;
- a decrease in weight gain was observed in animals vaccinated at 25 days of age (Fig. 1), varying according to the vaccine dose given. The sharpest decrease was observed in the groups vaccinated with the lowest dose (d1). The decrease in weight gain was less in rabbits vaccin ated with the highest dose (d2) and was observed later than in the other groups.

Figure 2 : Weight gain of young rabbits vaccinated at 29 days of age with the precocious line of *Eimeria magna*



T: rabbits born of naive does ; I : rabbits born of immunised vaccinated does
Vaccine doses : d1 = 3.5 x 10^3 oocysts or d2 = 3.5 x 10^4 oocysts.

- no decrease in weight gain was observed in animals vaccinated at 29 days of age (Fig. 2).

DISCUSSION-CONCLUSION

This trial using litters from immunised and naïve doe rabbits confirms that the doe's status does not modify the susceptibility of the litter to infection as already demonstrated by DROUET-VIARD *et al.*(1996).

The precocious line of *Eimeria magna* was able to protect young rabbits against a wild strain challenge inoculation when given to 25-day-old suckling rabbits, though it did not give full protection and the efficacy depended on the vaccine dose. Vaccination at 29 days of age with a dose as low as 3500 oocysts was totally effective. These preliminary results will be followed by other experiments with vaccination at intermediate ages and with lower vaccine doses.

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