VACCINATION AGAINST *EIMERIA MAGNA* COCCIDIOSIS USING SPRAY DISPERSION OF PRECOCIOUS LINE OOCYSTS INTO THE NEST BOX

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Abstract - Coccidiosis mostly affects young rabbits just after weaning (5-6-week-old animals). Prevention of this disease must therefore be initiated before weaning. "Precocious lines", derived from field species display good immunogenicity though being not pathogenic when administered at the right dose. In the present work, we tested the method of vaccination of the whole litter at 25 days of age by spray dispersion of oocysts of a precocious line of *Eimeria magna* in the nest box. Three doses were tested, d1= 4 x 10^2 oocysts, d2= 4 x 10^3 oocysts, d3= 4 x 10^4 oocysts. The animals were challenged with a wild strain of *E.magna* at 35 days of age, 5 days after weaning. The oocyst output and the weight gain were recorded after the vaccination and after challenge. The vaccinated animals did not display any vaccine reaction. The dose d3 was totally effective on the oocyst output and on the weight gain in our experimental conditions. As our challenge conditions were severe we think that a lower vaccine dose could be used. This method proved to be efficient, quick and easy to use because it did not require animal manipulation.

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. Lines with shortened cycles, so called "precocious lines", derived from field species display good immunogenicity though being not pathogenic when administered at the right dose. LICOIS *et al.* (1995) demonstrated the immunogenicity of a precocious line of *Eimeria magna* in young weaned rabbits. Sucklings in standard breeding conditions are not open to infection before the age of 20 days (ROSE, 1959; COUDERT et al 1991.) and they are fully sensitive at about 30 days of age corresponding to weaning age. In order to be effective vaccination should therefore be performed between 20 and 30 days of age. Experimentation aiming at the determination of the practical conditions for the use of these precocious lines to vaccine young rabbits, already shown that immunity acquired by the doe did not give protection to its litter (DROUET-VIARD et al 1994a) and that individual vaccination was possible as early as 25 days of age (DROUET-VIARD et al 1994b). In the present work, we tested the method of vaccination of the whole litter by spray diffusion of oocysts suspensions in the nest box. This method sparing animals manipulation would have great advantages.

MATERIAL AND METHOD

Animals. The young rabbits were the offspring from 11 litters of New Zealand white females from our coccidia free rabbitry (COUDERT et al 1988) housed in conventional conditions since their first gestation until their third gestation for this experiment. Housing conditions were those described by VIARD-DROUET et al (1983). All the does were fed a Robenidine supplemented commercial pelleted feed. The supplemented feed was replaced by non-supplemented pelleted feed three days before vaccination, (UAR 91360 Villemoison/Orge, France).

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.

a/ vaccination - Eleven litters were vaccinated at 25 days of age, by spraying 2 ml of an oocysts suspension of the precocious line of *E. magna* in the nest box, but not on the animals which were all temporarily outside the nest box; 3 litters were vaccinated with d1= 4 x 10^2 oocysts, 4 litters with d2= 4 x 10^3 oocysts, and 4 litters with d3= 4 x 10^4 oocysts .

Table 1 : Repartition of the weanlings in the experimental groups (G)

Each cage contained 3 animals. The vaccine doses correspond to the number of oocysts of the precocious line sprayed in the nest box. The challenge was performed individually per os with 10^4 oocysts of a wild strain of *E.magna*.

	Vaccine doses			
	none	d1= 4 x 10^2	d2= 4 x 10^3	d3= 4 x 10^4
unchallenged	G1 (3 cages)	/	G2 (2 cages)	G3 (2 cages)
challenged	G4 (3 cages)	G5 (4 cages)	G6 (8 cages)	G7 (8 cages)

Eighteen coccidia-free young rabbits born of 3 does in our SPF breeding unit, were used as non vaccinated control animals.
All the young were weaned when 30 days old. The rabbits were randomly affected to the different groups as represented in table 1.

b/ challenge inoculation - An individual challenge inoculation per os with 10^4 oocysts of the wild strain of *E.magna* was performed 5 days after weaning. Three groups were used as unchallenged controls.
The whole oocyst output was measured in each group between day 5 and day 10 after vaccination and between day 7 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 AND DISCUSSION

1/ Oocyst output after vaccination and after challenge inoculation.

Table 2: Individual total oocyst output after vaccination and after challenge inoculation.

The threshold of detection for the method used is 10^2 oocysts/g of feces.

* In 2/4 cages (G5) or 2/8 cages (G7), the excretion was undetectable (nd) and was not included in the mean.

Groups	Vaccination	Excretion after vaccination	Excretion after challenge
G1	none	nd	/
G2	d2	1.5 x 10^6	/
G3	d3	9 x 10^6	/
G4	none	nd	1.2 x 10^8
G5	d1	* 6 x 10^5	1.1 x 10^8
G6	d2	1.9 x 10^6	1.1 x 10^8
G7	d3	8.7 x 10^6	* 1.5 x 10^6

After vaccination (Table 2), all the animals multiplied the precocious line (excretion around 10^6 oocysts). We know that a rabbit inoculated with 1 oocyst of the precocious line produces about 3 x 10^3 oocysts, and that at these inoculation doses, the multiplication rate is proportional to the dose given (COUDERT, 1989 and COUDERT *et al.* 1995). Therefore, we can roughly estimate the number of oocysts ingested by each group: in groups vaccinated with d1, d2, and d3, the excretion corresponds respectively to an average effectively ingested vaccine dose of 50, 700, and 3000 oocysts.

There were variations in the excretions especially with the low doses sprayed. This is not surprising as the number of oocysts swallowed by each suckling can be very variable.

After the challenge inoculation, the oocyst output observed in groups 5 and 6 vaccinated with doses d1 and d2, corresponds to the maximum of excretion that can be observed in a rabbit inoculated with *E.magna*, whatever a given dose over 80 oocysts. A strong decrease in the output of oocysts was recorded in the group 7 vaccinated with the highest dose of 4×10^4 oocysts; the excretion after challenge was below 10^6 oocysts, so we considered that rabbits had acquired a good protection as this production is equivalent to the multiplication of one oocyst.

2/ Weight gain patterns

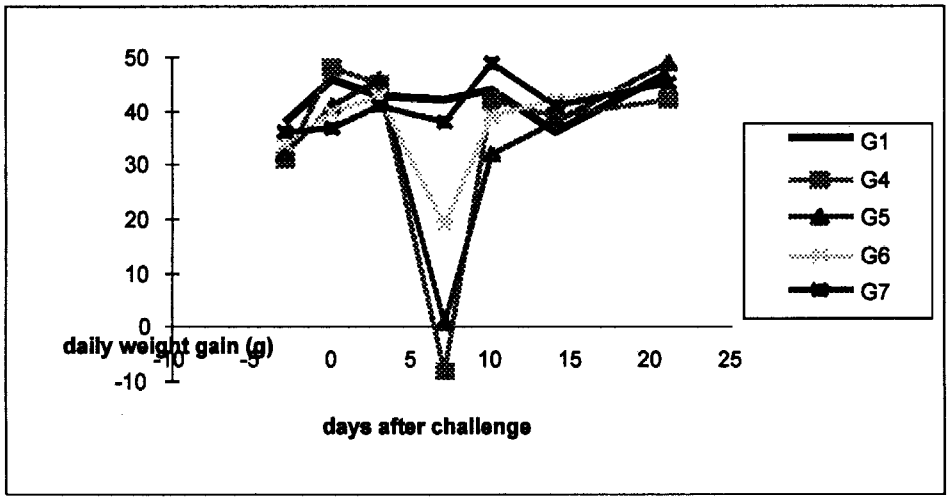
a) Weight gain after vaccination (figure 1) - After vaccination we could not see any difference in the weight gain of groups 6 and 7 which received only the precocious line, compared to that of group 1 (controls never inoculated). These results confirm a previous work (LICOIS et al 1995) in which we demonstrated the absence of pathogenicity of the precocious line at the doses used in this experiment.

b) Weight gain after challenge inoculation (figure 1) - The weight gain pattern after challenge inoculation was significantly different according to the experimental group ($P<0.1\%$):
- non-vaccinated control animals lost weight, the minimum being observed 7 days after the challenge;
- in the vaccinated groups, the sharpest decrease was observed with the lowest dose (d1), the decrease in weight gain was less in rabbits vaccinated with dose (d2), and no decrease was observed in animals vaccinated with the highest dose (d3).

So, considering this parameter for estimating acquired protection, litters vaccinated with 4×10^4 oocysts were totally protected.

Figure 1 : Daily weight gain patterns in groups of rabbits vaccinated at 25 days of age by spray dispersion of oocysts of *Eimeria.magna* precocious line in the nest box.

Three vaccine doses were tested: G5: 4×10^2 oocysts, G6: 4×10^3 oocysts, G7: 4×10^4 oocysts. G1: non vaccinated unchallenged group. G4: non vaccinated challenged group. The daily weight gains patterns of G2 and G3 were not different from those of G7 and G1 and are not represented.



CONCLUSION

A "spray vaccination" with a precocious line at 25 days of age, did not induce any vaccine reaction, because the dose of oocysts actually ingested by the sucklings was low. Despite these low doses ingested, a spray of oocysts in the nest box is able to protect young rabbits against a wild strain challenge inoculation. The efficacy of the protection depends on the vaccine dose; a « spray vaccination » with $4x\ 10^4$ oocysts of the precocious line of *Eimeria magna* is totally effective. In the present experiment, there were only 5 days between the vaccination and weaning, and 10 days between vaccination and challenge. Because the challenge conditions were rather severe, we think that in the field, we could use lower spray doses, especially in the context of a weaning at 35 days, when animals would have more time to get immunised.

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REFERENCES

COUDERT P., 1989. Some peculiarities of rabbit coccidiosis. in Coccidia and intestinal coccidiomorphs, *Vth International Coccidiosis Conference, Tours (France), 17-20 october 1989. Ed. INRA Publ.*

COUDERT P., LICOIS D., BESNARD J., 1988. Establishment of a Specified Pathogen Free breeding colony (SPF) without hysterectomy and hand-rearing procedures. In: *Proceedings of the 4th Congress of the World Rabbit Science Association, October 10-14, Budapest,* 137-148.

COUDERT P., NACIRI M., DROUET-VIARD F., LICOIS D., 1991. Mammalian coccidiosis natural resistance of suckling rabbits. *2d Conference Cost-Action 1989. 2-5 April, Münchenwiler, Suisse.*

COUDERT P., LICOIS D., DROUET-VIARD F. 1995. Eimeria and Isospora. Eimeria species of rabbits. in: *Biotechnology. Guidelines on Techniques in Coccidiosis Research. (Eckert J., Braun R., Shirley M.W., Coudert P., Ed). pp 52-73. Office for official publications of the European communities. Luxembourg.*

DROUET-VIARD F., COUDERT P., ROUX C., LICOIS D., BOIVIN M., 1994a. Etude de l'immunité transmise aux lapereaux par des femelles immunisées contre *Eimeria magna.* In: *VIèmes Journées de la Recherche Cunicole, 6-7 déc 1994, La Rochelle, Vol 1,* 45-52

DROUET-VIARD F., COUDERT P., LICOIS D., BOIVIN M., 1994b. Essai de vaccination contre une coccidiose *a Eimeria magna* à l'aide d'une lignée précoce. In: *VIèmes Journées de la Recherche Cunicole, 6-7 déc 1994, La Rochelle,* **Vol 1,** 39-43.

LICOIS D., COUDERT P., DROUET-VIARD F., BOIVIN M., 1995. Eimeria magna: Pathogenicity, immunogenicity and selection of a precocious line. *Veterinary Parasitology,* **60,** 27-35.

PEETERS J., GEEROMS R., VAREWYCK H., BOUQUET Y., LAMPO P., HALEN P. 1983. Immunity and effect of clopidol/methylbenzoquate and robenidine before and after weaning on rabbit coccidiosis in the field. *Research in Veterinary Science.* **35,** 211-216.

ROSE ME. (1959). PhD thesis, University of Cambridge.

VIARD-DROUET F., COUDERT P., DURAND P., PROVOT F. 1983. Pathology of breeding rabbit does. Evolution of several plasma parameters in primiparous rabbit does. *Ann. Rech. Vét.,* **14 (2),** 105-115.

Résumé: Vaccination contre la coccidiose a *eimeria magna* par pulverisation d'oocystes de la lignee precoce dans la boite a nid.

La coccidiose affecte particulièrement les lapereaux sevrés âgés de 5 à 6 semaines. La prévention de cette maladie doit donc être initiée avant le sevrage. Des "lignées précoces", dérivées des espèces rencontrées sur le terrain, ont montré un bon pouvoir immunogène tout en étant apathogènes lorsqu'elles sont utilisées à dose convenable. Dans ce travail, nous avons testé la possibilité de vacciner toute la portée à l'âge de 25 jours par pulvérisation d'oocystes de la lignée précoce d'*Eimeria magna* dans la boite à nid. Trois doses ont été testées, d1= 4×10^2 oocystes, d2= 4×10^3 oocystes, d3= 4×10^4 oocystes. Les animaux ont reçu une inoculation d'épreuve de 10^4 oocystes d'une souche sauvage d'*Eimeria magna* à l'âge de 35 jours soit 5 jours après le sevrage. L'excrétion d'oocystes et le gain de poids ont été enregistrés après la vaccination et après l'inoculation d'épreuve. Les animaux vaccinés n'ont eu aucune réaction vaccinale. Dans nos conditions expérimentales, la dose vaccinale d3 s'est révélée totalement efficace après l'inoculation d'épreuve, tant sur l'excrétion d'oocystes que sur le gain de poids. Les conditions d'épreuve étant sévères, une dose vaccinale plus faible pourrait probablement être utilisée avec succès. Cette méthode s'est révélée efficace, rapide et facile à utiliser puisqu'elle ne nécessite pas de manipulation des animaux.