



# Vaccination of Algerian Local Rabbits with Precocious Strains of *Eimeria magna* and *Eimeria media*

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## ABSTRACT

The present study was conducted to assess the safety and the efficacy of a vaccine containing the Algerian precocious strains of *Eimeria magna* and *Eimeria media* used separately or together against rabbit coccidiosis. The samples consisted of 56 young rabbits reared in specific pathogen-free conditions. Following the challenge inoculation, statistically significant decreases in oocyst excretion were noticed in the vaccinated rabbits with the precocious strain of *Eimeria magna*, *Eimeria media*, and both species leading to a good immune response acquired by the vaccination associated with a good growth rate. Moreover, there was a statistically significant increase in oocyst output following the challenge in all challenged groups. Unlike the vaccinated groups, the challenged groups showed poor weight gains. More than 50% of the young rabbits from all the challenged groups presented diarrhea. Consequently, these precocious strains constitute good candidates for mono or polyvalent anticoccidial vaccines in the future.

**Keywords:** Precocious strain, Rabbits, Vaccination, Wild strain.

## INTRODUCTION

Coccidiosis is recognized as one of the major handicaps in rabbit breeding (Cowie-Whitney, 1977). It causes considerable economic losses due to the decrease in weight gain, diarrhea, and even death (Drouet-Viard et al., 1997a; Drouet-Viard et al., 1997b). All domesticated rabbit breeds can be infected by coccidia, especially the younger animals aged one to four months (Drouet-Viard et al., 1997a; González-Redondo et al., 2008; Bachene et al., 2018). For a long time, the control of rabbit coccidiosis was based on continuous administration of anticoccidial drugs in feed or drinking water. The use of anticoccidial drugs resulted in drug-resistant problems in rabbit farming (Coudert et al., 1988; Peeters et al., 1988). Inoculation with live attenuated *Eimeria* parasites could provide sufficient protection against the challenges with the corresponding wild strains (Akpo et al., 2012; Bachene et al., 2018) and provides a practical way to develop the attenuated vaccine against rabbit coccidiosis. In Algeria, some studies have reported the epidemiological status of coccidiosis in rabbits, showing that *E. magna* and *E. media* are the predominant species of *Eimeria* (Henneb and Aissi, 2013; Maziz-bettahar et al., 2018). Therefore, in the present study both precocious strains of *E. magna* and *E. media* were tested separately and together to evaluate and compare the parental strains in terms of their safety and efficacy as vaccine strains against rabbit coccidiosis.

## MATERIAL AND METHODS

### Ethical approval

The current study was approved by the scientific council of the higher National Veterinary School of Algiers, Algeria.

### Parasites

#### Wild strains of *Eimeria magna* and *Eimeria media*

The wild strains of *Eimeria* spp. were isolated in Algeria in 2014 from local rabbits and inoculated to coccidia-free rabbits, then purified and preserved in 2.5% potassium dichromate solution at 4°C.

#### Precocious strain of *Eimeria magna*

Precocious strain of *E. magna* obtained from previous study (Bachene et al., 2018) in Algeria in 2016, was used in the current study. Briefly, the precocious strain of *E. magna* was obtained from the corresponding Algerian wild strain by the selection of the early oocyst excretion after nine passages in coccidia-free rabbits and preserved in 2.5% potassium dichromate solution at 4°C.

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### **Precocious strain of *Eimeria media***

A precocious strain of *E. media* was obtained in Algeria in 2016 from the corresponding Algerian wild strain by the selection of the early oocyst excretion after 13 passages in coccidia-free rabbits according to the method described by Licois et al. (1990). Table 1 summarizes the selection process of precocious strain of *E. media*. This strain was preserved in 2.5 % potassium dichromate solution at 4°C.

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

Inoculated strain	Inoculation dose	Time interval between inoculation and collection of the first oocysts from fecal contents (hours)	Obtained strain
WS.Emed2014	10 <sup>4</sup> oocysts	108	S1
S1	2 x 10 <sup>4</sup> oocysts	102	S2
S2	3 x 10 <sup>4</sup> oocysts	96	S3
S3	5 x 10 <sup>4</sup> oocysts	92	S4
S4	5 x 10 <sup>4</sup> oocysts	90	S5
S5	5 x 10 <sup>4</sup> oocysts	90 <sup>a</sup>	S6
S6	5 x 10 <sup>4</sup> oocysts	90	S7
S7	5 x 10 <sup>4</sup> oocysts	90	S8
S8	5 x 10 <sup>4</sup> oocysts	90	S9
S9	5 x 10 <sup>4</sup> oocysts	88	S10
S10	5 x 10 <sup>4</sup> oocysts	84	S11
S11	10 <sup>5</sup> oocysts	78	S12
S12	10 <sup>5</sup> oocysts	72	PS.Emed 2016

WS.Emed2014: Wild strain of *Eimeria media* obtained in 2014. PS.Emed 2016: Precocious strain of *Eimeria media* obtained in 2016.

### **Experimental design**

The samples consisted of 56 young rabbits reared in specific pathogen-free conditions as described by Coudert et al. (1988). All the young rabbits were fed *ad libitum* with a commercial pelleted food free from anticoccidial drugs. They were weaned at 25 days old and divided into 7 groups (8 rabbits per group). The NNC group was associated with non-vaccinated non-inoculated control group, the NCma group referred to non-vaccinated, challenged with the wild strain of *E. magna*, the NCme group referred to non-vaccinated, challenged with the wild strain of *E. media*. Moreover, NCma group dealt with non-vaccinated, challenged with the wild strains of *E. magna* and *E. media*, the VCma group addressed vaccinated with the precocious strain of *E. magna*, challenged with the wild strain of *E. magna*, the VCme group included vaccinated cases with the precocious strain of *E. media*, challenged with the wild strain of *E. media*, and finally VCma group entailed vaccinated cases with the precocious strains of *E. media* and *E. magna*, challenged with both corresponding wild strains.

At 27 days of age, each rabbit in the vaccinated groups received the corresponding *Eimeria* spp. orally at a dose of  $2.5 \times 10^3$  oocysts/0.2 ml of distilled water. The vaccinated group with both *Eimeria* spp. received half of the above dose of each. At 40 days of age, each rabbit in challenged groups was orally inoculated with corresponding *Eimeria* spp. at a dose of  $5 \times 10^4$  oocysts/0.2 ml of distilled water. The inoculated group with both *Eimeria* spp. received half of the above dose of each.

### **Fecal sampling and parasitological analysis**

Feces were daily collected from each group from day 2 post-vaccination to day 21 post-challenge. Oocysts counting was performed as described by Coudert et al. (1995).

### **Rabbit monitoring**

The animals were weighed once every 3 days for 32 days. Animals were examined to detect diarrhea or any other abnormalities.

### **Statistical analysis**

Statistical analysis was performed by one-way ANOVA post hoc multiple comparisons using the SPSS software (version 17). The oocyst excretion and means of average weights per time across the seven studied groups were compared and the difference between groups was considered statistically significant when p-values were less than 0.05.

## **RESULTS**

### **Oocysts output after vaccination and challenge inoculation**

In the vaccinated group with the precocious strain of *E. magna*, total excretion throughout the experiment period was  $22.4 \times 10^6$  oocysts per rabbit. After the challenge inoculation (13 days post-vaccination) the oocyst excretion decreased significantly with a percentage of 98.4%, compared to the unvaccinated group. In the vaccinated group with

the precocious strain of *E. media*, total excretion throughout the experiment period was  $36.8 \times 10^6$  oocysts. After the challenge inoculation (13 days post-vaccination) the oocyst excretion decreased significantly with a percentage of 98.9%, compared to the unvaccinated group. Vaccinated rabbits with the mixture of both precocious strains of *E. magna* and *E. media* multiplied these parasites with a total excretion of  $28.4 \times 10^6$  oocysts. After the challenge inoculation (13 days post-vaccination) the oocyst excretion decreased significantly with a percentage of 99.4%, compared to the unvaccinated group. There was no significant difference between the vaccinated groups in terms of oocysts output reduction (Table 2).

### Average weight gains per time

The control group showed regular growth throughout the experiment. The vaccinated groups (VCma, VCme, VCmame) indicated good growth during the experiment with acceptable weight gains (Figure 1) compared to the control group ( $p < 0.05$ ).

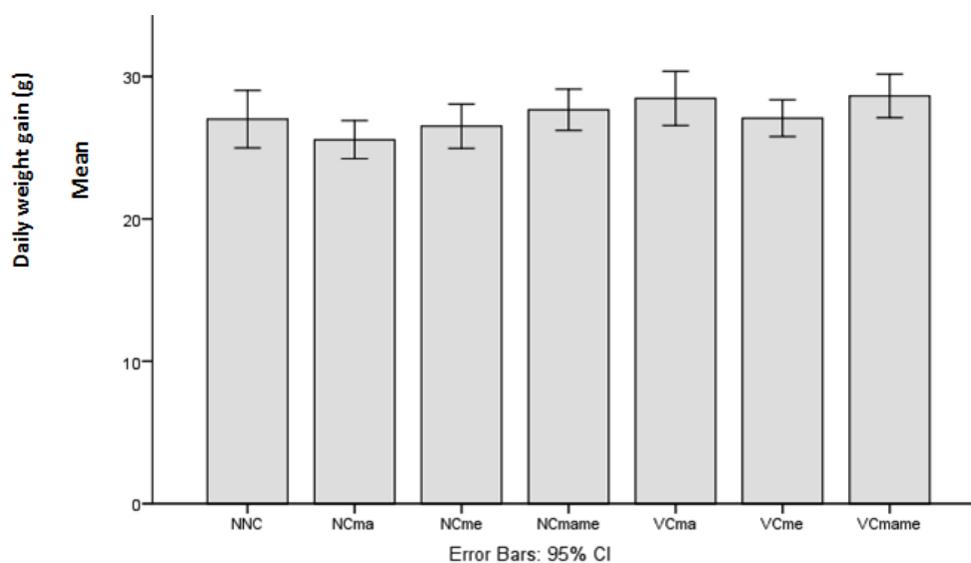
### Mortality and clinical symptoms

No mortality occurred in any group throughout the experiment. No case of diarrhea was noticed in the vaccinated-challenged groups (VCma, VCme, VCmame) as well as the control one (NNC). However, some cases of diarrhea occurred in all challenged non-vaccinated groups. For instance, three rabbits from the NCma group showed diarrhea 7-10 days after the challenge and two others from the NCme group suffered from diarrhea 6-9 days after the challenge. In NCmame group, diarrhea was noticed in 6 rabbits during a period of 6-10 days post-inoculation.

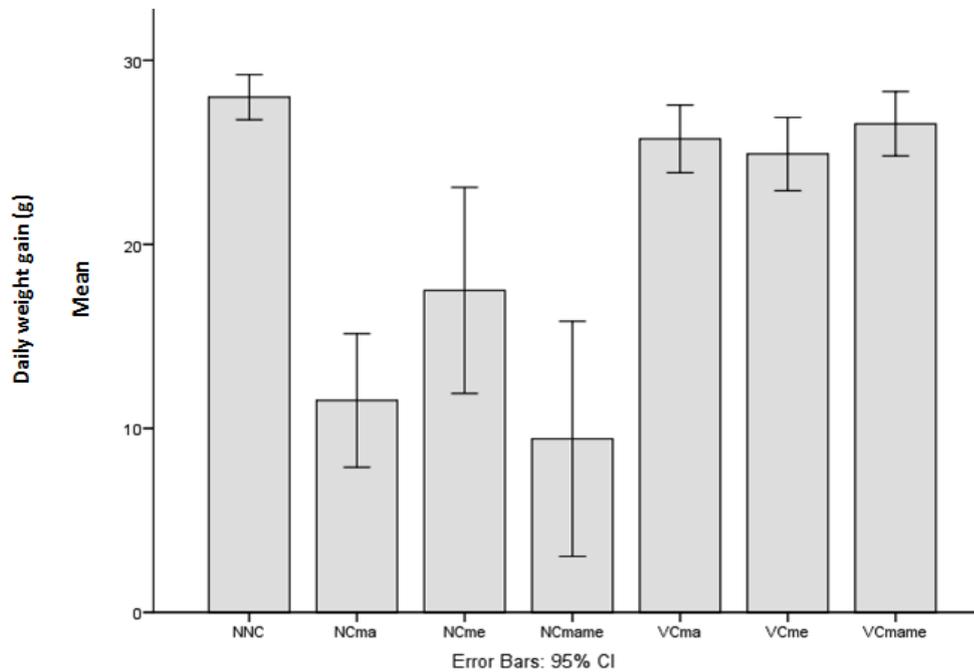
**Table 2.** Total oocysts output per rabbit after vaccination and challenge infection with *Eimeria* species.

Groups	Oocyst output		Reduced percentage of oocyst excretion in vaccinated groups compared with unvaccinated groups
	After vaccination	After challenge	
NNC	-	-	-
NCma	-	$341.4 \times 10^6$	-
NCme	-	$395.5 \times 10^6$	-
NCmame	-	$898.4 \times 10^6$	-
VCma	$22.4 \times 10^6$	$5.4 \times 10^6$	98.4%
VCme	$36.8 \times 10^6$	$4.4 \times 10^6$	98.9%
VCmame	$28.4 \times 10^6$	$5.2 \times 10^6$	99.4%

NNC: Non-vaccinated, non-challenged control group; NCma: Non-vaccinated, challenged with the wild strain of *E. magna*; NCme: Non-vaccinated, challenged with the wild strain of *E. media*; NCmame: Non-vaccinated, challenged with the wild strains of *E. magna* and *E. media*; VCma: Vaccinated with the precocious strain of *E. magna*- challenged with the wild strain of *E. magna*; VCme: Vaccinated with the precocious strain of *E. media*- challenged with the wild strain of *E. media*; VCmame: Vaccinated with the precocious strains of *E. media* and *E. magna*, challenged with the corresponding wild strains.



**Figure 1.** Average daily weight gains post-vaccination in the seven studied groups. NNC: Non-vaccinated non challenged control group. NCma: Non-vaccinated – challenged with the wild strain of *E. magna*. NCme: Non-vaccinated – challenged with the wild strain of *E. media*. NCmame: Non-vaccinated – challenged with the wild strains of *E. magna* and *E. media*. VCma: Vaccinated with the precocious strain of *E. magna*- challenged with the wild strain of *E. magna*. VCme: Vaccinated with the precocious strain of *E. media*- challenged with the wild strain of *E. media*. VCmame: Vaccinated with the precocious strains of *E. media* and *E. magna*-challenged with the corresponding wild strains. The challenged groups (NCma, NCme, NCmame) showed low weight gains, compared to vaccinated and control groups (Figure 2).



**Figure 2.** Average daily weight gains post-challenge infection in the seven studied groups. NNC: Non-vaccinated non-challenged control group. NCma: Non-vaccinated – challenged with the wild strain of *E magna*. NCme: Non-vaccinated – challenged with the wild strain of *E media*. NCmame: Non vaccinated – challenged with the wild strains of *E magna* and *E media*. VCma: vaccinated with the precocious strain of *E magna*- challenged with the wild strain of *E magna*. VCme: vaccinated with the precocious strain of *E media*- challenged with the wild strain of *E media*. VCmame: vaccinated with the precocious strains of *E media* and *E magna*- challenged with the corresponding wild strains.

## DISCUSSION

*E. magna* and *E. media* are considered as mildly pathogenic (Licois et al., 1995), and are also the most predominant *Eimeria* species in rabbit breeding (Coudert et al., 1988; Licois et al., 1995; HennebandAissi, 2013; Maziz-Bettahar et al., 2018; Bachene et al., 2019). The mentioned strains were used in the current study separately or together as a live attenuated vaccine for rabbits. Following vaccination, the precocious strains of *E. magna* and *E. media* reproduced in young rabbits (VCma, VCme, VCmame) without expressing the disease, testifying to the viability of these strains and the attenuation of their pathogenicity. Indeed, vaccination could protect rabbits against challenge infection. In the present experiment, a dose of  $2.5 \times 10^3$  oocysts of precocious strains of *E. magna*, *E. media*, or both strains led to the partial protection of the investigated samples. In fact, the results indicated a decrease in oocyst output of about 98.4%, 98.9%, and 99.4% in VCma, VCme, VCmame groups, respectively compared with challenged non-vaccinated groups. However, the non-vaccinated, challenged groups with the wild strains of *E. magna*, *E. media* separately or together displayed diarrhea. There was no statistically significant difference between the vaccinated groups with a single species or with the two species together, this can encourage the use of polyvalent vaccines against rabbit coccidiosis since the natural infections with a single *Eimeria* species are rare as reported previously (Jing et al., 2012; Abdel-Baki and Al-Quraishy, 2013). The result demonstrated the attenuation of *E. magna* and *E. media* precocious strain's pathogenicity is globally in agreement with the results of Licois et al. (1995), Pakandl et al. (1996), Drouet-Viard et al. (1997a, 1997b, 1997c), and Bachene et al. (2018).

## CONCLUSION

The Algerian precocious strains of *Eimeria magna* and *E. media* were less pathogenic, compared to the wild strains. On the other hand, their effectiveness has been verified by the challenge inoculation using Algerian wild strains of *E. magna* and *E. media* leading to a satisfactory result in terms of acquired protection. Consequently, the findings indicated the absence of mortality and morbidity as well as a decrease in oocyst output after the challenge inoculation. Vaccination seems to be efficient when performed when rabbits are as old as 27 days giving time to young rabbits to develop sufficient immunity at weaning. Given these results, these precocious strains of *E. magna* and *E. media* appear to be suitable candidates for use as live attenuated single or polyvalent vaccines in rabbit breeding.

## DECLARATIONS

### Competing interests

The authors declare that they have no conflict of interests.

### Authors' contributions

MSB proposed and conducted the study. MSB and AB drafted and revised the manuscript. ST and HA supervised the work. MSB and AB analyzed the data. All authors read and approved the final manuscript.

## REFERENCES

- Abdel-Baki AAS, and Al-Quraishy S (2013). Prevalence of coccidia (*Eimeriaspp.*) infection in domestic rabbits, *Oryctolagus cuniculus*, in Riyadh, Saudi Arabia. *Pakistan Journal of Zoology*, 45(5): 1329-1333. Available at: [http://zsp.com.pk/pdf45/1329-1333%2020%20PJZ-1386-13%2019-9-13%20Revised%20MS%201\\_.pdf](http://zsp.com.pk/pdf45/1329-1333%2020%20PJZ-1386-13%2019-9-13%20Revised%20MS%201_.pdf)
- Akpo Y, Kpodékon MT, Djago Y, Licois D, and Youssao IA (2012). Vaccination of rabbits against coccidiosis using precocious lines of *Eimeriamagna* and *Eimeria media* in Benin. *Veterinary Parasitology* 184: 73-76. DOI: <https://doi.org/10.1016/j.vetpar.2011.08.012>
- Bachene MS, Temim S, Ainbaziz H, Bachene A and Suo X (2018). A vaccination trial with a precocious line of *Eimeria magna* in Algerian local rabbits *Oryctolagus cuniculus*. *Veterinary Parasitology*, 261: 73-76. DOI: <https://doi.org/10.1016/j.vetpar.2018.08.013>
- Coudert P, Licois D, and Drouet-Viard F (1995). *Eimeria* species and strains of rabbits. In: Eckert, J., Braun, R., Shirley, M.W., Coudert, P, Sc (Eds.), *Biotechnology Guidelines on Techniques in coccidiosis Research*. European commission, Luxembourg, pp. 52-73. Available at: <http://ndl.ethernet.edu.et/handle/123456789/47174>
- Coudert P, Licois D, and Besnard J (1988). Establishment of a specified pathogen free breeding colony (SPF) without hysterectomy and hand-rearing procedures. *Proceedings of the 4th Congress of the World Rabbit Science Association*, pp. 137-148. Available at: <http://world-rabbit-science.com/WRSA-Proceedings/Congress-1988-Budapest/communications-pdf/G08-COUDERT.pdf>
- Cowie-Whitney J (1977). Diseases of the commercial rabbit. *Veterinary Record*, 101: 299-303. DOI: <https://doi.org/10.1136/vr.101.15.299>
- Drouet-Viard F, Coudert P, Licois D, and Boivin M (1997a). Vaccination against *Eimeria magnacoccidiosis* using spray dispersion of precocious line oocysts in the nest box. *Veterinary Parasitology* 70: 61-66. DOI: [https://www.doi.org/10.1016/S0304-4017\(96\)01134-X](https://www.doi.org/10.1016/S0304-4017(96)01134-X)
- Drouet-viard F, Coudert P, Licois D, and Boivin M (1997b). Vaccination trial against *Eimeria magnacoccidiosis* using a precocious line. *World Rabbit Science*, 5(2): 51-54. DOI: [https://www.doi.org/10.1016/S0304-4017\(96\)01133-8](https://www.doi.org/10.1016/S0304-4017(96)01133-8)
- Drouet-viard F, Coudert P, Licois D, and BoivinM(1997c). Acquired protection of the rabbit (*Oryctolagus cuniculus*) against coccidiosis using a precocious line of *Eimeria magna*: effect of vaccine dose and age at vaccination. *Veterinary Parasitology*, 69: 197-201. DOI: [https://www.doi.org/10.1016/S0304-4017\(96\)01133-8](https://www.doi.org/10.1016/S0304-4017(96)01133-8)
- González-Redondo P, Finzi A, Negretti P, and Micci M (2008). Incidence of coccidiosis in different rabbit keeping systems. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 60(5): 1267-1270. DOI: <https://www.doi.org/10.1590/S0102-09352008000500034>
- Henneb M, and Aissi M (2013). Etude cinétique de l'excrétion oocystale chez la lapine et sa descendance et identification des différentes espèces de coccidies. 15 th day of research about rabbits, November 19 th – 20 th France, pp. 221-224. Available at: <http://www.cuniculture.info/Docs/Magazine/Magazine2013/fichiers-pdf-JRC/P02-Henneb.pdf>
- Jing F, Yin GW, Liu XY, Suo X, and Qin YH (2012). Large-scale survey of the prevalence of *Eimeria* infections in domestic rabbits in China. *Parasitology Research*, 110(4): 1495-1500. DOI: <https://doi.org/10.1007/s00436-011-2653-4>
- Licois D, Coudert P, Boivin M, Drouet-Viard F, and Provot F (1990). Selection and characterization of a precocious line of *Eimeria intestinalis*, an intestinal rabbit coccidium. *Parasitology Research*, 76: 192-198. DOI: <https://www.doi.org/10.1007/BF00930814>
- Licois D, Coudert P, Drouet-Viard F, and Boivin M (1995). *Eimeria magna*: pathogenicity, immunogenicity and selection of a precocious line. *Veterinary Parasitology*, 60: 27-35. DOI: [https://www.doi.org/10.1016/0304-4017\(94\)00768-8](https://www.doi.org/10.1016/0304-4017(94)00768-8)
- Maziz-Bettahar S, Aissi M, Ainbaziz H, Bachene MS, Zenia S, and Ghisani F (2018). Prevalence of coccidian infection in rabbit farms in North Algeria. *Veterinary World*, 11(11): 1569-1573. DOI: <https://www.doi.org/10.14202/vet.world.2018.1569-1573>
- Pakandl M, Eid Ahmed N, Licois D, and Coudert P (1996). *Eimeria magan* Pérard: Study of the endogenous development of parental and precocious strains. *Veterinary Parasitology*, 65: 213-222. DOI: [https://www.doi.org/10.1016/s0304-4017\(96\)00975-2](https://www.doi.org/10.1016/s0304-4017(96)00975-2)
- Peeters JE, Geeroms R, and Halen P (1988). Evolution of coccidial infection in commercial and domestic rabbits between 1982 and 1986. *Veterinary Parasitology*, 29: 327-331. DOI: [https://www.doi.org/10.1016/0304-4017\(88\)90149-5](https://www.doi.org/10.1016/0304-4017(88)90149-5)