2021, Scienceline Publication
World's Veterinary Journal
World Vet J, 11(1): 45-50, March 25, 2021
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2021.wvj6

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

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ORIGINAL ARTICLE
pii: S232245682100006-11
Received: 24 Dec 2020
Accepted: 11 Feb 2021

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 toa 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 coccidiosisinrabbits,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.

To cite this paper: Bachene MS, Temim S, Ainbaziz H, and Bachene A (2021). Vaccination of Algerian Local Rabbits with Precocious Strains of *Eimeria magna* and *Eimeria media*. *World Vet. J.*, 11 (1): 45-50.

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	10^4 oocysts	108	S1
S1	2 x 10^4 oocysts	102	S2
S2	3 x 10^4 oocysts	96	S3
S3	5 x10^4oocysts	92	S4
S4	5 x10^4oocysts	90	S5
S5	5 x10^4oocysts	90[a]	S6
S6	5 x10^4oocysts	90	S7
S7	5 x 10^4oocysts	90	S8
S8	5 x 10^4oocysts	90	S9
S9	5 x 10^4oocysts	88	S10
S10	5 x 10^4oocysts	84	S11
S11	10^5 oocysts	78	S12
S12	10^5 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 conditionsas 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 associatedwithnon-vaccinated non-inoculated control group, the NCma group referred to non-vaccinated, challenged with the wild strain of *E. magna*, the NCmesignifiednon-vaccinated, challenged with the wild strain of *E media*. Moreover, NCmame group dealt withnon-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 includedvaccinated cases with the precocious strain of *E. media*, challenged with the wild strain of *E media*, and finally VCmame group entailedvaccinatedcases 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 2.5×10^3oocysts/ 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 5×10^4oocysts/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 (version17). 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 22.4×10^6 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

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the precocious strain of *E. media*, total excretion throughout the experiment period was 36.8×10^6 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 28.4×10^6 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	-	341.4×10^6	-
NCme	-	395.5×10^6	-
NCmame	-	898.4×10^6	-
VCma	22.4×10^6	5.4×10^6	98.4%
VCme	36.8×10^6	4.4×10^6	98.9%
VCmame	28.4×10^6	5.2×10^6	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.

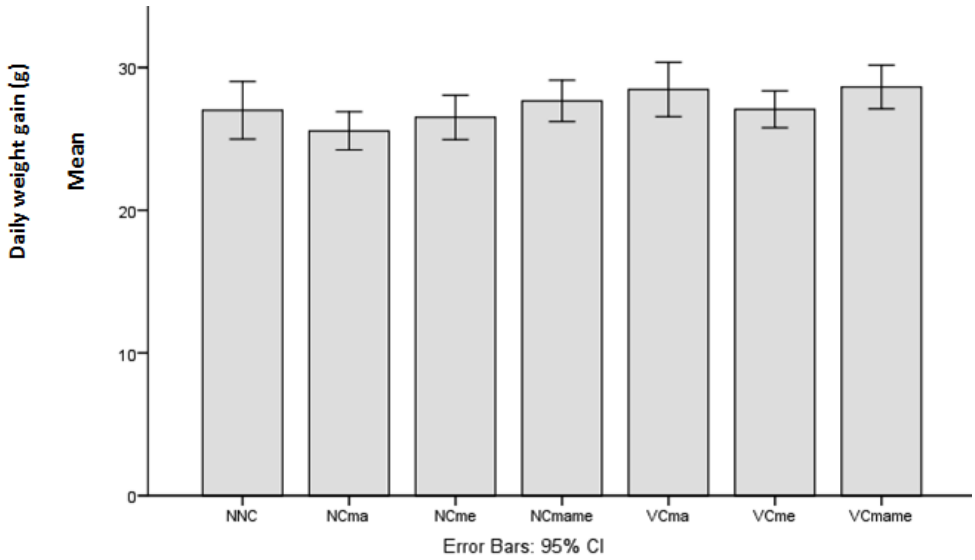

Figure 1. Average daily weight gains post-vaccination in the seven studied groups. NNC: Non-vaccinated non challenged control group. NCma: Non-vaccinated – challenged with the wild strain of *E magna.* NCme: Non-vaccinated – challenged with the wild strain of *E media.* NCmame: Non-vaccinated – challenged 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).

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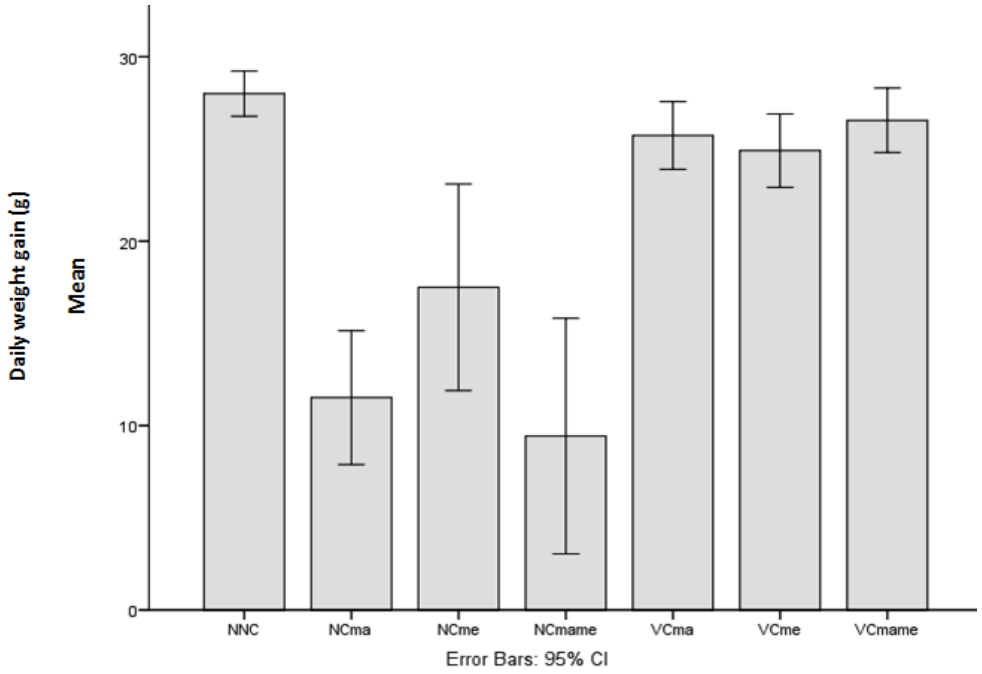

Figure 2. Average daily weight gains post-challenge infection in the seven studied groups.NNC: Non-vaccinated non challenged control group. NCma: Non-vaccinated – challenged with the wild strain of *E magna*. NCme: Non-vaccinated – challenged with the wild strain of *E media*. NCmame: Non vaccinated – 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 2.5×10^3oocysts 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, Vcmamegroups, 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 Bacheneet 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.

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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.

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