Effect of exposure pre-weaning bunnies to sporulated Eimeria oocysts on immunity and growth traits

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Abstract
Coccidiosis is the most serious protozoan parasites affecting young rabbits just after weaning (5-6 weeks old). It causes large economic losses for rabbit industry especially in case of severe infection. Suckling bunnies at 25 days of age were vaccinated by spraying dispersion of naturally attenuated strain of *Eimeria* oocysts mixture in the nest box with 3 doses (40, 400 and 4000 oocysts per bunny). The animals were challenged individually orally with $5x10^3$ oocysts of mixture of *Eimeria* species at 35 days old. The oocysts output, body weight and mortality were recorded after vaccination and after challenge. Vaccination reduced oocysts output after challenge inoculation and prevented the decrease in weight gain observed in non vaccinated challenged animals. This method of vaccination (spray) gives the opportunity to be efficient, quick and easy and avoid animal manipulation.

Key words: *Eimeria, vaccination, rabbit.*

Introduction

In fattening rabbit industry, digestive disorders are the main causes of morbidity and mortality and cause large economic losses. Among the specific causes of the pathological changes, parasites (coccidia) and some bacteria mainly enteropathogenic *Escherichia coli* are predominant. The clinical condition generally observed is characterized by anorexia and reduced live and carcass weights, caused mainly by decreased food intake over the first four weeks. The condition may progress to death, which is attributed to the hepatic lesions that are also associated with the infection **(Gomez - bautista *et al.*, 1986)**. In the study conducted by **Freitas *et al.* (2009)**, infection due to *E. stiedai* caused ascites, hepatomegaly associated with congestion and fibrosis, thickening of the bladder wall and retention of bile.

Drouet-Viard *et al.* (1997) noted that, coccidiosis mostly affects young rabbits just after weaning (5 to 6 week old). Prevention of this disease must therefore be initiated before weaning. 'Precocious lines', derived from field species display good immunogenicity, though not pathogenic when administered at the right dose. **Homouda (1995)** declared that, the most dangerous period is the first 3 weeks after the transfer of animals from single boxes at about the age of 10 weeks. He mentioned that the infection rate may reach 100 percent of these feedlots and up to 50% of the animals may develop clinical coccidiosis with a mortality rate of 30 percent.

Licois *et al.* (1995) demonstrated the immunogenicity of a developed earlier line of *E. magna* in young weaned rabbits. Suckling in standard breeding conditions are not susceptible to infection before the age of 20 days**,** and they are fully sensitive at about 30 days old corresponding to weaning age **(Coudert *et al.*, 1991)**. In order to be effective, vaccination should therefore be performed between 20 and 30 days old. **Drouet-Viard *et al.* (1997a)** in a similar study found that age of 25 days was the best age to vaccinate the bunnies.

Pakandl (2009) mentioned that vaccination trials were preformed by **Drouet-Viard *et al.* (1997b)** with a precocious line of *E. magna*. Vac-cination either *per os* or using spray dispersion of oocysts into nest boxes gave satisfactory results. He added that development of efficacious methods, alternative to continuous medication with anticoccidial drug, of rab-bit coccidiosis control, is a challenge for future research work.

The aim of this work is to determine if this method of vaccination against coccidiosis is able to be used under Egyptian practical conditions with the study of some bunnies' growth traits.

Materials and Methods

The practical part of this study was carried out in a private farm, while all lab procedures were carried out at the Animal Health Research Institute (AHRI), Parasitology Department.

Animals:

In this study 25 coccidia free female doe Newzeland white rabbits (NZW) with their off springs were randomly divided into five groups with 20 to 25 bunnies per group. Doe rabbits were housed individually in wire cages (55 x 50 x 45 cm). They fed with a commercial pelleted rabbit ration *ad libitum* with free access to fresh water through watering nipples. Nest box measures were 45x35x40 cm.

Offspring of groups 1, 2 and 3 were vaccinated once by spraying the attenuated *Eimeria spp*. oocysts mixture in the birth nest at 25 days of age. Vaccination dose was equal to 40, 400 and 4000 oocysts per bunny. Groups 4 and 5 were not vaccinated.

Bunnies of groups 1, 2, 3 and 4 received orally a challenged inoculation with $5x10^3$ *Eimeria spp*. oocysts. The 4th group was none vaccinated but received a challenge dose, while the 5th group was none vaccinated and non challenged (control).

Parasites:

The *Eimeria spp*. oocysts for vaccination and for challenge inoculation were obtained from group of naturally infected rabbits and recently sporulated. They were kept at 4°C in 2.5 % potassium dichromate solution and were classified and identified according to **Soulsby (1988)**. The method used for counting the number of oocysts per ml. was described by **Caudert *et al.* (1995).**

The vaccination and challenge contained a mixture of *E. intestinalis, E. coecicola, E. magna, E. media* and *E. stiedai.*

Vaccination:

Nest boxes of vaccinated groups were sprayed with 2ml. of saline contain 40, 400 and 4000 oocysts (the bunnies were out of the box) (**Drouet-Viard *et al.*, 1997a)**.

Output oocysts:

The output oocysts were measured in each group between 5 and 9 days after vaccination and between 4 and 10 days after challenge inoculation according to **Drouet-Viard *et al.* (1997b)**.

Productive performance:

Bunnies' weight and mortality were recorded during the study.

Statistical analysis:

Data were statistically analyzed using Least Square Means, according to **SAS (1996)** using the following model:

$$\mathbf{Y_{jj} = m + T_i + e_{jj}}$$

Where :

Y_{ij} = Observation of the i^{th} doe rabbit.

m = Overall mean, common element to all observations.

T_j = Effect of treatment group (i=1..5)

e_{jj}= Random error component assumed to be normally distributed.

Results

Oocysts output:

Table (1) shows the oocysts output after vaccination and after inoculation. The excretion of oocysts of the vaccine line was checked 9 days after vaccination. All the animals multiplied the vaccine line (excretion over 7.1×10^6). Therefore, after challenge inoculation, a decrease in the output of oocysts was recorded in all vaccinated groups. In rabbits vaccinated with 40 oocysts/bunny there was clearly detectable oocysts output.

After the challenge inoculation, the oocysts output observed in groups1 (vaccinated with 40 oocysts/bunny) and group 4 (non vaccinated) challenged. No oocysts excretions were detected in groups 4 and 5.

Table (1). Effect of vaccination in suckling bunnies on *Eimeria species* oocysts ex-

Group	No. of Bunnies	Vaccination Dose/ bunny	Excretion after vaccine	Challenge dose/bunny At age of 35 days	Excretion after challenge
G1	22	40vaccinationon ation and after inoculation	$1.2\times10^{6\ c}$	5000	$20000^{\ b}$
G2	23	400vaccin ation and after inoculation	$5.6\times10^{6\ b}$	5000	$3000^{\ b}$
G3	22	4000vaccin ation and after inoculation	$7.1\times10^{6\ a}$	5000	$Non^{\ b}$
G4	25	Non vaccination	$Non^{\ d\ *}$	5000	$1.7\times10^{7\ a}$
G5	21	Non vaccination	$Non^{\ d\ *}$	$Non^{\ **}$	$Non^{\ b\ *}$

*: No vaccination *: Less than 100 oocysts were detected.
a, b, c, d: values in the same column with different superscript differ significantly

Weight gain after vaccination:

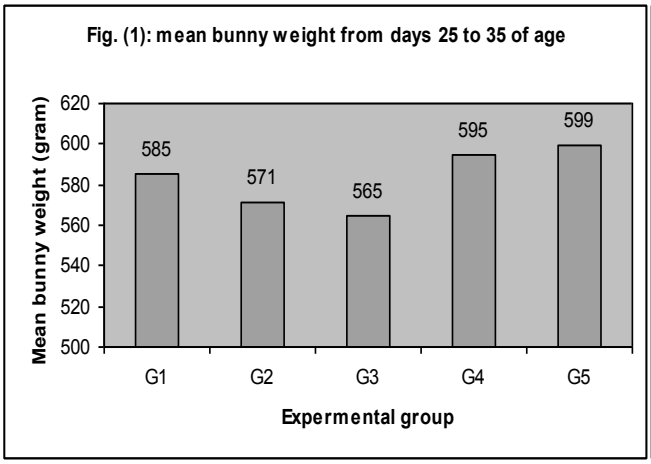
Table (2). Mean bunny weight at different stages of the experiment

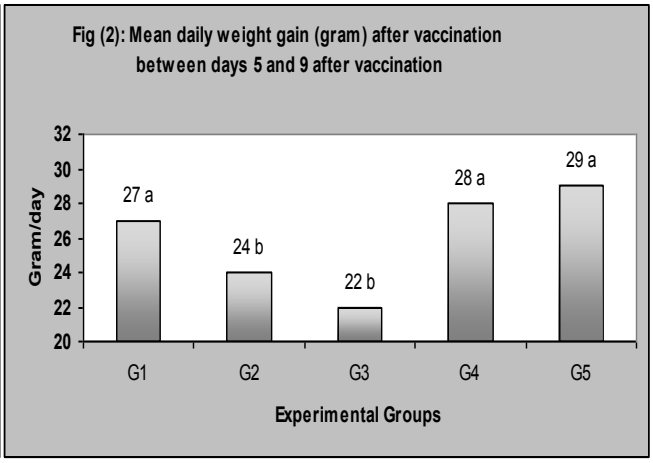
STAGE	GROUPS					
	G1	G2	G3	G4	G5	P
S1	585±36.0	571±35.2	565± 36.0	595±33.8	599±36.8	P > 0.05
S2	27 ± 1.88^{a}	24 ± 1.84^{b}	22 ± 1.88^{b}	28 ± 1.77^{a}	29 ± 1.92^{a}	P < 0.05
S3	15 ± 1.74^{b}	25 ± 1.66^{a}	27 ± 1.70^{a}	12 ± 1.74^{b}	29 ± 1.74^{a}	P < 0.05

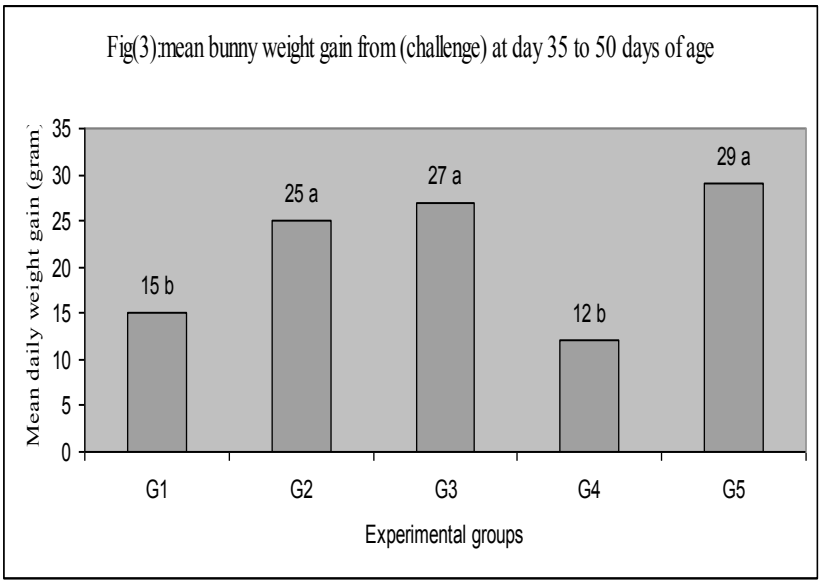
S1: Mean bunny weight at 35 days of age after vaccination at 25 days.
S2: Mean bunny weight gain between days 5 and 9 after vaccination at 25 days of age.
S3: Mean bunny weight gain after challenge at 35 days and at 50 days of age.
G1: bunnies vaccinated by spraying nest box with 40 oocysts/bunny then challenged orally with 5000 oocysts at age of 35 days.
G2: bunnies vaccinated by spraying nest box with 400 oocysts/bunny then challenged orally with 5000 oocysts at age of 35 days.
G1: bunnies vaccinated by spraying nest box with 4000 oocysts/bunny then challenged orally with 5000 oocysts at age of 35 days.
G4: bunnies not vaccinated but challenged orally with 5000 oocysts at age of 35 days.
G5: bunnies not vaccinated or challenged. (control)
a, b: values in the same row with different superscript differ significantly.

The mean bunny weights of the all vaccinated groups G1 (585±36.0 g), G2 (571±35.2 g) and G3 (565± 36.0 g) were not significantly different (P > 0.05) from mean bunny weight of not vaccinated groups G4 (595±33.8 g) and G5 (599±36.8 g) throughout day 25 (start) to day 35 (challenge) (Fig.1).

However, mean bunny weights gain of G2 (24 ± 1.84^{b} g) and G3 (22 ± 1.88^{b} g) were significantly lower (P<0.05) between day 5 and day 9 after vaccination, compared to that of the other three groups G1 (27 ± 1.88^{a} g), G4 (28 ± 1.77^{a} g) and G5 (29 ± 1.92^{a} g) Fig. (2). Bunnies of the vaccinated G3 displayed a vaccine reaction, but without diarrhea or mortality.







Weight gain after challenge inoculation:

The weight gain after challenge inoculation at 35 day till 50 days of age was significantly higher in the vaccinated groups G2 (25±1.66[a] g) and G3 (27±1.70[a] g) and control G5 (29±1.74[a] g) than in the vaccinated G1 (15±1.74[b] g) and non-vaccinated groups G4 (12±1.74[b] g) $P<0.05$ (Fig. 3) this happened because 6 bunnies of vaccinated (G1) and 9 bunnies of non vaccinated (G4) lost weight due to diarrhea (Fig. 3).

Mortality:

After challenge, diarrhea causes death of one bunny in G1 and two bunnies in G4.

DISCUSSION

All the oocysts sprayed in the nest box were ingested; each rabbit ingested 20-25% of the dose to a maximum (5 or 4 suckling per nest box) according to their oocysts excretion post spray. There were variations in excretion. This is not surprising as the number of oocyst effectively swallowed by each suckling can be very variable. Moreover, it was clear that a rabbit inoculated with one oocyst of the precocious line produces about 3×10^3 oocysts and that, at these inoculation doses, the multiplication rate is proportional to the dose given (**Coudert, 1989 and Coudert *et al*., 1995).** The vaccination by naturally strain of *E. steadi* oocyst was able to protect weanlings against challenge inoculation, whatever the age at vaccination was (25, 27, 29 days of age) **(Drouet-Viard *et al.* 1997a).**

In this study, a dose of 30000 oocysts gave total protection and no oocysts output was de-

tectable. This is due to development of strong immunity against challenge. So, it's believed that rabbits had acquired good protection, as this production is equivalent to the multiplication of one oocysts. These results are parallel to that recorded by **Drouet -Viard *et al*. (1994)** who found that a dose of 3500 oocysts gave total protection to suckling vaccinated at 25, 27, or 29 days old . At the same manner **Akpo *et al.* (2012)** in Benin found that a dose of 5×10^3 oocysts of the 2 precocious lines of *E. magna* and *E. media* confirming a good immunogenic characteristic of the precocious lines and therefore constitute good potential candidates for vaccine production for this country.

Drouet-Viard *et al*. (1994) observed that, at 25-days – old animals challenged 9 days after vaccination, not completely protected against coccidiosis, but in this trial with a challenge performed 13 days post vaccination there was total protection. These differences in results may be attributed to the time allowed for acquisition of complete immunity which was not long enough.

Regarding with oocysts output after vaccination, all the animals multiplied the vaccine line (excretion over 7.1×10^6) (Table 1). This result was different from ***Akpo et al (2012)*** who found that over $(24 \pm 2.6) \times 10^6$ oocysts per animal were obtained in both vaccinated groups**.**

The present results reveals that the mean bunny weights of the all vaccinated groups G1, G2 and G3 were not significantly different ($P > 0.05$) from mean bunny weight of not vaccinated groups G4 and G5 from day 25 (start) till day 35 (challenge) (Table.2).

The mean bunny weights gain of G2 and G3 were significantly lower ($P<0.05$) between day 5 and day 9 after vaccination, compared to that of the other three groups G1, G4 and G5 figure (2). However, the weight gain after challenge inoculation was significantly higher in the vaccinated G2 and G3 and control G5 than in the vaccinated G1 and non-vaccinated G4 ($P < 0.05$) (Fig. 3) .these results were parallel to **Akpo *et al.* (2012)** who found that, the weight gains of the two vaccinated groups were not significantly different ($P >0.05$) throughout the experiment and significantly lower ($P < 0.01$) in the vaccinated groups, between day 5 till day 10 after vaccination, compared to that of the control ones. The weight gain after challenge inoculation was significantly higher in the vaccinated group than in the non-vaccinated group ($P < 0.05$).

The safety of these vaccine lines demonstrated by **Akpo *et al*. (2010)** associated with their immunogenicity made them potential candidates as vaccines against intestinal rabbit coccidiosis.

Conclusion

The used method of vaccination (spray) reduced significantly the oocysts output after challenge inoculation and led to a correct growth. It gave the impression to be efficient, quick, easy and avoid animal manipulation. These results give an opportunity the vaccination of an important population of rabbits in the field.

References

Akpo, Y., Kpodékon, M., Djago, Y., Licois, D. and Youssao, I.A.K. (2010). Evaluation of inoculation of rabbit using precocious lines of Eimeria magna and Eimeria media. Actes Atelier International sur valorisation des programmes Corus et Aires sud en sciences médicales, pharmaceutiques et vétérinaires: maladies parasitaires, bactériennes, génétiques ouvirales d'intérêt médical et vétérinaire en Afrique du 22–26 Novembre.Bobo-Dioulasso: CIRDES.

Akpo,Y.; Kpodékon, M.T., Djago Y., Licois D. and Youssao, I.A.K. (2012). Vaccination of rabbits against coccidiosis using precocious lines of Eimeria magna and Eimeria media in Benin Veterinary Parasitology 184 : 73– 76.

Coudert, P. (1989). Some peculiarities of rabbit coccidiosis. In: Coccidia and Intestinal Coccidiomorphs. Proceeding of the 5th International Coccidiosis Conference., 17-20 October, Tours (France), pp.: 481- 488.

Caudert, P., D.Licois and J. Besnard (1991). Establishment of a specified pathogen free

breeding colony (SPF) without hysterectomy and hand-rearing procedures. Proceeding of the 4th Congress of the World Rabbit Science Association. 10-14 October 1988, Budapest, pp.: 137-148.

Coudert, P., D. Licois and Drouet-Viard,F. (1995). Eimeria and Isospora. Eimeria species of rabbit. In: J. Eckert, R. Braun, M. W. Shirley and P. Coudert (Editors). Biotechnology. Gudidelines on Techniques in Coccidiosis Research.Office for Official Publications of European Communities, Luxembourg, pp.: 52-73.

Drouet-Viard, F., P. Couplasert, P. Durand and F. Provot (1983). Pathology of breeding rabbit does. Evaluation of several plasma parameters in primiparous rabbit does. Ann. Rech. Vet., 14: 105-115.

Drouet-Viard,F., Coudert, P., Licois, D. and Boivin, M., (1994). Essai de vaccination contre une coccidiosis à Eimeria magna à Íaide d`une lignée précoce In: Vlémes Journées de la Recherche Cunicole,6-7 dés 1994, La Rochelle, Vol 1, pp.39-43.

Drouet-Viard F., Coudert, P, Licois, D. and Boivin, M., (1997a). 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.

Drouet-Viard F., Coudert, P, Coudert, P. and Boivin,M., (1997b). Vaccination against Eimeria magna coccidiosis using spray dispersion of precocious line oocysts in the nest box. Veterinary Parasitology 70: 61-66

Freitas,F. L.daC., Yamamoto, B. L., Freitas, W.L.daC., Almeida, K. de S., Alessi, A. C., Machado, R. Z., Machado, C. R. (2009). Pathological and biochemical aspects of the hepatic coccidiosis in rabbits. Revista de Patologia Tropical, v. 38, n. 2, p. 115-125.

Gomez-Bautista, M.; Garcia, M.V. and Rojo -Vazquez, F.A. (1986). The levels of total protein fractions in the serum of rabbits infected with Eimeria stiedai. Annales de Parasitologie Humaine et Comparée, v. 61, n. 4, p. 393-400,.

Homouda, I.A. (1995). Effect of some herbal growth promoter on calcium absorption in femals rabbit "J. Agric. Sci., Mansoura Univ., 20 (7). 3199-3205.

Licois, D., P. Coudert, F. Drouet-Viard and M. Boivin (1995). Eimeria magna: Pathogenicity, immunogenicity and selection of a precocious line. Vet. Parasitol. 60:27-35

Pakandl, M. (2009). Coccidia of rabbit: **a review.** Folia Parasitologica 56[3]:153–166.

SAS. (1996). SAS' Procedure Guide. Version 6.12, Forth Edition, SAS Institute, Inc., Carry, NC, USA.

Soulsby, E. J. L. (1988). Helminth, Arthopods and Protozoa of Domestic Animals. English Language Book Society, Baillere, Trindal.

تأثير تعريض الأرانب قبل الفطام لبويضات الكوكسيديا علي المناعة ومعدلات النمو

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الملخص العربي

الكوكسيديا من أخطر أنوع الطفيليات التى تؤثر على معدلات النمو فى الحيوانات وبخاصة الارانب . يظهر الأثر السيئ للكوكسيديا في الأرانب الصغيرة عادة بعد الفطام (عمر 5-6 أسابيع). تسبب الكوكسيديا خسائر اقتصادية ضخمة في حالة الإصاباتة الحادة. فى هذه الدراسة رش بيت الولادة (فارغ) لأرانب غير مفطومة عند عمر 25 يوم بخليط من خمس عترات من الكوكسيديا بمعدل 40، 400 و 4000 بويضة لكل أرنب .ثم تم اعطاء جرعة تحدي بواسطة تجريع الأرانب المفطومة عند عمر 35 يوم بجرعة تحتوي على 5000 بويضة من نفس تركيبة جرعة التحصين.

تم تسجيل عدد البويضات المفرزة ، وزن الجسم، معدل النفوق بعد كل من جرعتي التحصين والتحدي. أظهرت النتائج أن التحصين قلل من عدد البويضات الكوكسيديا المفرزة وقلل من فقد الوزن نتيجة الاصابة بالكوكسيديا.

من النتائج المتحصلة من التجربة تبين أن طريقة تحصين الأرانب ضد الكوكسيديا بواسطة رش بيت الولادة تبدو فعالة، سريعة، بسيطة وبدون التعامل مباشرة مع الأرانب.