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EFFECT OF EXPOSURE PRE-WEANING BUNNIES TO SPORULATED EIMERIA OOCYSTS ON IMMUNITY AND GROWTH TRAITS OF RABBITS

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This experiment aimed to determine if spraying intestinal sporulated Eimeria oocysts in an empty nest box will give natural immunity to growing bunnies against serious invocation comparing to the effect of rabbit ration pellets admixed with anti-coccidian powder under Egyptian practical conditions. Twenty five coccidia free New Zealand White (NZW) does with their offspring were randomly divided equally into 5 groups (G1 to G5) with mean weight of 455.2±10.69 gram/bunny. At day 25 of bunnies' age, 15 ml of saline contain equal mixed of four intestinal Eimeria spp. oocysts (40, 400 or 4000 per bunny) were sprayed in empty nest boxes of groups 1, 2 and 3 respectively (vaccination). At day 35 bunnies of all five groups were challenged orally with 5 ml of saline contained 5000 of equal mixed inoculation Eimeria spp. oocysts (challenge), the experiment ended at day 45 of bunnies' age. All groups were fed to ad libitum with a rabbit commercial pellet ration, but only group 5 ration was supplemented with anti coccidian powder.

Results obtained indicated that post vaccination high oocysts excretions were observed combined with mortality rates and diarrhea in G1, G2 and G3.

Post challenge high oocysts excretions were observed combined with high mortality rates and diarrhea in G1, G2 and G4 (G4 was the highest), while G3 and G5 had no oocysts excretions.

Feed intake and feed conversion between days 35-45 of age had significant differences between some groups where G5 was the best.

***Conclusively,** the results make us to advice to keep using the anti-coccidian in ration until we solve the problem of mortality post*

vaccination and to do more studies to confirm or deny our present results under the Egyptian conditions.

Key words: Rabbits, *Eimeria*, oocysts excretion, mortality, weight

In rabbit industry, getting infected by intestinal Eimeria species (coccidosis) causes digestive disorders, in some cases severe intestinal morbidity and mortality symptoms occurs, Leysen *et al.* (1989) noted coccidia is responsible for major losses in rabbit industry. The clinical condition generally observed is characterized by reduced in both food intake and body weight, also, diarrhea 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).

Drouet-Viard *et al*. (1997b) noted 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. Also, Homouda (1995) 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 is 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) found that age of 25 days was the best age to vaccinate the bunnies and full protection was obtained.

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

Therefore, the aim of this work is to determine if spraying intestinal sporulated Eimeria oocysts in an empty nest box will give natural immunity to growing bunnies against serious invocation comparing to the effect of rabbit ration pellets admixed with anti coccidian powder, under Egyptian practical conditions.

MATERIALS AND METHODS

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

Animals:

Twenty five coccidia free New Zealand White (NZW) does rabbits with their offspring were randomly divided equally into 5 groups (G1 to G5) with 26 to 28 bunnies per group with mean weight 455.2±10.69 gram. Doe rabbits were housed individually in wire cages 45x55x35 cm, the nest box measures were 35x35x35 cm. At day 35 bunnies were weaned by transferring the doe to another cage and bunnies of each cage were weighed.

Feeding:

Bunnies of groups 1 to 5 were fed to *ad libitum* with a rabbit commercial pellet ration, but only group 5 ration was supplemented with anti coccidiosis powder approved and used in Europe (European Medicines Agency). Free access to fresh water through watering nipples was granted.

Parasites:

The *Eimeria spp*. oocysts for vaccination and challenge inoculation were obtained from naturally infected rabbits and recently sporulated, it contained a mixture of *E. intestinalis* (very pathogenic), *E. magna* (moderate pathogenic) and *E. coecicola* and *E. media* (non or slightly pathogenic) as classified by Lebas *et al.* (1997).

The oocysts 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).

Vaccination and challenge:

- At day 25 of age bunnies of groups 1, 2 and 3 were vaccinated with equal mixed *Eimeria spp.* oocysts by spraying 15 ml of saline contain 40, 400 and 4000 oocysts per bunny of groups 1, 2 and 3 respectively (the bunnies were out of the box) (Drouet-Viard *et al.*, 1997a).

- At day 35 bunnies of all groups were challenged orally with 5 ml of saline contained 5000 of equal mixed inoculation *Eimeria spp*. oocysts. Vaccination and challenge step were summarized at Table 1:

Table 1: Groups treatments (vaccination) : at day 25 of age bunnies were exposed to sporulated Eimeria oocysts by spraying it into empty nest box, (challenge): at day 35 of age were orally challenged.

Items	**Treatment groups**				
	G1	**G2**	**G3**	**G4**	**G5**
Vaccination (day 25)	yes	yes	yes	no	no
oocysts/bunny (spray)	40	400	4000	0	0
Challenge (day35)	yes	yes	yes	yes	yes
Oocysts/bunny (orally)	5000	5000	5000	5000	5000

Output oocysts:

The output oocysts were measured from bunnies feces (collected by butting a plastic net under the cages) 6-9 days post vaccination and challenge inoculation according to Drouet-Viard *et al.* (1997b).

Productive performance:

Bunnies' numbers and weights at days 25 (vaccination), 35 (challenge) and 45 (the end) were recorded. Also, feed intake between days 35-45 was recorded.

Bunnies' means of weight gain and mortality rate% between days 25-35, 35-45 and 25-45 were calculated. Feed intake, feed conversion between days 35-45 were also calculated.

***Statistical analysis*:**

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

$$\mathbf{Y_{ij} = \mu + T_i + F_j + e_{ijk}}$$

Where: Y_{ijk} = Observation of the i^{th} doe rabbit, μ = Overall mean, common element to all observations, T_j = Effect of treatment group (i=1..5), F_j = Feed without or with anti-coccdian powder (j= 1 & 2), e_{ijk} = Random error component assumed to be normally distributed.

Differences among means were tested by Duncan's multiple range test (Duncan, 1955). P.S.: the effect of $\mathbf{F_j}$ (Feed without/with anti-coccidian

powder) was considered in the statistical analysis model just to remove its effect on the standard error.

RESULTS:

Oocysts excretion and mortality ratio%:

Oocysts excretion:

At day-35 of age (10 days post vaccination) oocysts excretions were 1.2×10^6, 5.6×10^6, 7.1×10^6, ND (not detectable) and ND for groups 1 to 5, respectively. While, at day-45 of age (10 days post challenge) oocysts excretions were 2×10^5, 3×10^4, ND, 1.7×10^7 and ND for groups 1 to 5, respectively (Table 2).

Table 2: Effect of exposure bunnies to sporulated Eimeria oocysts by spraying them at day 25 into empty nest box (vaccination) and then orally challenge at day 35 on oocysts excretion, bunnies number and mortality rate, %.

Items		Treatment groups					SE	Sig
		G1	G2	G3	G4	G5		
Oocysts excretion at d-35		$1.2\times10^{6\,c}$	$5.6\times10^{6\,b}$	$7.1\times10^{6\,a}$	nd ^{d}	nd ^{d}	4.3×10^5	**
Oocysts excretion at d-45		$2\times10^{5\,b}$	$3\times10^{4\,c}$	nd ^{d}	$1.7\times10^{7\,a}$	nd ^{d}	4.0×10^5	**
	Age (days)							
Bunny Number	**25**	27	28	26	27	26	0.30	**
	35	25 ^{ab}	25 ^{ab}	22 ^{b}	27 ^{a}	26 ^{ab}	0.30	**
	45	21 ^{ab}	22 ^{ab}	21 ^{ab}	19 ^{b}	25 ^{a}	0.21	**
Mortality Rate %	**d-25 to d-35**	7.41 ^{c}	10.71 ^{b}	15.38 ^{a}	0.00 ^{d}	0.00 ^{d}	0.15	*
	d-35 to d-45	16.00 ^{b}	12.00 ^{c}	4.55 ^{d}	29.63 ^{a}	3.85 ^{d}	0.37	*
	d-25 to d-45	22.22 ^{b}	21.43 ^{b}	19.23 ^{c}	29.63 ^{a}	3.85 ^{d}	0.56	*

A, b, c, d: Means in the same column, with different letters, differ significantly.

** : $P\leq 0.01$

Mortality ratio,%:

Mortality rates between days 25-35 of age were 7.41%, 10.71%, 15.38%, 0.00% and 0.00% for groups 1 to 5, respectively. While, mortality

between days 35-45 of age rates were 16.0%, 12.0%, 4.55%, 29.63% and 3.85% for groups 1 to 5, respectively (Table 2). Overall mortality rates between days 25-45 of age were 22.22%, 21.43%, 19.23%, 29.63% and 3.85% for groups 1 to 5, respectively.

Some growth performance traits and feed intake:

At day-35 of age (10 days post vaccination), mean bunny body weights of the vaccinated groups (G1, G2 and G3) were lower to significantly lower comparing with non vaccinated groups (G4 and G5) means were 596.6, 576.4, 598.6, 609.4 and 617.0 grams, respectively. Also, the same remark was appeared in mean body weight gain trait (table 3).

At day-45 of age (10 days post challenge), significant differences between all groups in means of final body weight and weight gain. Means of final bunny weights were 700.2, 717.0, 769.0, 691.2 and 846.4 grams. While, means of body weight gain were 242.2, 264.8, 309.4, 250.0 and 405.2 grams for groups 1 to 5, respectively (Table 3).

Feed intake and feed conversion between days 35-45 of age had significant differences between some groups. Feed intake means were 484.7, 578.0, 765.5, 495.0 and 814.6 grams. While, feed conversion ratios were 4.37, 4.11, 4.43, 4.86 and 3.56 for groups 1 to 5, respectively (Table 3).

Table 3: Means of bunny weight (gm), weight gain (gm), feed in take (gm) and feed conversion from 25 -45 days of age.

Age (days)	Bunny Weight (gm)			Weight Gain (gm)			Feed intake	Feed conversion
	25	35	45	d-25 to d-35	d-35 to d-45	d-25 to d-45	d-35 to d-45	d-35 to d-45
Groups								
G1	464.2^{a}	596.6^{b}	700.2^{cd}	132.6^{bc}	110.0^{d}	242.2^{d}	484.7^{b}	4.37^{ab}
G2	452.4^{ab}	576.4^{c}	717.0^{c}	124.2^{c}	140.6^{c}	264.8^{c}	578.0^{b}	4.11^{ab}
G3	459.6^{a}	598.6^{b}	769.0^{b}	137.2^{bc}	172.2^{b}	309.4^{b}	765.5^{a}	4.43^{ab}
G4	460.4^{a}	609.4^{ab}	691.2^{d}	149.0^{b}	101.0^{d}	250.0^{cd}	495.0^{b}	4.86^{a}
G5	441.4^{b}	617.0^{a}	846.4^{a}	175.8^{a}	229.4^{a}	405.2^{a}	814.6^{a}	3.56^{b}
SE	**4.78**	**5.12**	**6.93**	**5.86**	**5.43**	**7.08**	**52.79**	**0.28**
	**	**	**	**	**	**	**	**

abcd: Means in the same column, with different letters, differ significantly.

** : $P \leq 0.01$

DISCUSSION:

According to Coudert *et al.* (1995) who stated that intestinal coccidia can produce 1-5×10^6 per digested oocysts, we can from the results of secreted oocysts estimate that only 1-2% of the sprayed oocysts were ingested.

In this study, only vaccination dose of 4000 oocysts (G3) gave an execration of 7.1×10^6 oocysts which is lower than those recorded by Akpo et al (2012) who found that over (24±2.6)×10^6 oocysts per animal, but this caused a mortality rate of 15.38% and that may be caused due to the noticeable diarrhea which occurred on vaccinated bunnies. On the other hand, this dose (4000 oocysts) gave a total protection when bunnies were challenged and no oocysts output were detected post challenge (table 2), these results are in agreement 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. This result is believed that bunnies had acquired good protection and developed strong immunity against infection with coccidia.

Post vaccination, a high mortality rates and low body weight gains (tables 2 and 3) were observed accompanied with appearance of diarrhea in groups 1, 2 and 3 bunnies which caused economical losses especially in G3 comparing with groups 4 and 5, this result indicates that the possible reason is the vaccination and the increase of mortality rate came in harmony with the dose sprayed.

Also, higher mortality rates and lower body weight gain accompanied with appearance of diarrhea appeared again after challenge doses were given at day 35, but this time in groups 1, 2 and 4 comparing with groups 3 and 5 and caused again high economical losses especially in G4.

The previous results came in harmony with Leysen *et al*. (1989) who noted coccidia is responsible for major losses in rabbit industry. On the other hand, Akpo *et al.* (2012) recorded no mortality or diarrhea after vaccination.

Also, the previous results indicates that the vaccination given previously to the bunnies of group 3 protected them from the harmful effect of the challenge dose, which came in harmony with Drouet-Viard *et al.* (1997a), and the anti-coccidian powder which admixed with group 5 ration gave the same protection.

The low performance in means of bunny weight, weight gain and feed intake in challenged groups 1, 2 and 4 post challenge (days 35-45) may be caused as a result of low appetite and diarrhea occurred on sick bunnies

which came in agreement with Leysen *et al*. (1989) and poor feed conversion resulted because the total feed intake was related to live bunnies at the time of challenge-included the feed consumed by dead bunnies.

Conclusion:

In this study, vaccination (spray the empty nest poxes with 4000 oocysts per bunny) reduced significantly the oocysts output after challenge inoculation and led to a correct growth. It gave the impression to be efficient, quick, and easy and avoid animal manipulation. However, the high mortality rate post vaccination led to high economical losses.

These results make us to advice to keep using the anti-coccidian in ration until we solve the problem of mortality post vaccination and advice for more studies to confirm or deny our present results under the Egyptian conditions.

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تأثير رش خلفات الأرانب قبل الفطام لحويصلات الكوكسيديا المعدية على المناعة وبعض صفات النمو

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كان الهدف من هذه التجربة هو تقييم تأثير رش حويصلات كوكسيديا معوية معدية داخل بيوت ولادة خالية من الخلفات وما إذا كانت ستعطي مناعة طبيعية للخلفات ضد أي عدوى شديدة وذلك مقارنة بالمناعة المعطاة بواسطة مضاد كوكسيديا مضاف للعلف تحت الظروف العملية المصرية.

تم استخدام ٢٥ أنثى خالية من الكوكسيديا من سلالة النيوزيلندي الأبيض مع خلفاتها ، قسمت الإناث إلى خمس مجموعات متساوية بمتوسط وزن ٤٥٥،٢±١٠،٦٩ جرام لكل خلفة.

في اليوم ٢٥ من عمر الخلفات تم رش ١٥ مل من محلول ملحي يحتوي على أعداد متساوية من حويصلات أربعة سلالات من الكوكسيديا المعوية المعدية بمعدل ٤٠، ٤٠٠، ٤٠٠٠ حويصلة / خلفة للمجموعات ١ ، ٢ ، ٣ على التوالي (التحصين) واستبقت المجموعتين ٤ ، ٥ لجرعة التحدي.

في اليوم ٣٥ من عمر الخلفات تم إجراء عملية التحدي وذلك بتجريع كل خلفة ٥ مل محلول ملحي يحتوي على ٥٠٠٠ حويصلة معوية معدية من السلالات المستخدمة في الرش. وانتهت التجربة في عمر ٤٥ يوم من عمر الخلفات.

تم تغذية كل مجموعات الأرانب حتى الشبع على عليقة أرانب تجارية خالية من مضادات الكوكسيديا ماعدا المجموعة الخامسة فتمت لها اضافة مضاد كوكسديا للعليقة.

بعد جرعة التحصين ظهرت إفرازات من بويضات الكوكسيديا من خلفات المجموعات ١ ، ٢ ، ٣ مصحوبة بمعدلات نفوق وإسهال وكان أعلاها المجموعة الثالثة.

بعد جرعة التحدي ظهرت إفرازات من بويضات الكوكسيديا من خلفات المجموعات ١ ، ٢ ، ٤ مصحوبة بمعدلات نفوق وإسهال وكان أعلاها المجموعة الرابعة.

اختلفت معدلات الغذاء المأكول والتحويل الغذائي بين المجاميع وكان أفضلها المجموعة الخامسة.

التوصية: من هذه النتائج نوصي باستمرار إضافة مضادات الكوكسيديا للعليقة وإجراء مزيد من الأبحاث لتأكيد أو نفي هذه النتائج تحت الظروف المصرية.