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Effect of Eimeria stiedae infestation on the immune response of rabbit vaccinated with oil adjuvant polyvalent rabbit Pasteurellosis

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In an attempt to evaluate the possible role of *Eimeria stiedae* infection on rabbit vaccinated with haemorrhagic septicaemia oil adjuvant vaccine, a total of 60 New-Zealand rabbits were divided into 6 groups (A- F). The first four groups subdivided into two subgroups. The subgroups (A1, A2) vaccinated and infected at time of 1st dose of vaccine, subgroup (B1, B2) vaccinated and infected at 2 weeks post 1st vaccination, subgroup (C1, C2) which vaccinated and infected at the time of 2nd dose of vaccination, finally subgroup (D1, D2) vaccinated and infected at 2 weeks post 2nd dose of vaccine. Group E vaccinated only but the group F left as non vaccinated non infected (control). The results revealed that *E. stiedae* infection at the time or after 2 weeks from first or second dose of vaccination (A1, B1, C1 and D1) and treated with semduramycine 150 showed slight decrease of the antibody titer in contrast the untreated group (A2, B2, C2 and D2) showed sudden decrease of P. multocida antibody titer measured by indirect haemagglutination and ELISA test. Vaccinated group (E) was the superior one showing the highest antibody titer. The challenge test of all rabbit groups with virulent *P. multocida* revealed a protective percent of 83.4%, 50%, 100% and 0 % in treated, untreated, vaccinated and control group respectively, but subgroups C2, D2 the protective value was 33.4% this due to challenge concurrency post or at the time of infection. These findings reflect the important to avoid coccidial infection following vaccination programs to obtain better immune response to haemorrhagic septicaemia oil adjuvant pasteurellosis vaccine and high level of protection.

Many countries of the world including Egypt, rabbits represent as a good and acceptable source of animal protein and in addition to the fact that rabbit, breed mostly in large numbers and their offspring grow rapidly. Moreover, its skin has also an economic value for production, in addition it is one of the most important experimental animals (Sandford, 1986).

Rabbit meat as food is pearly white, poor in fat, high nutritious, easily digestible, and palatable, for these reasons rabbit meat is recommended for sick and convalescent people.

Any disease either bacterial or viral or parasitic could affect dramatically rabbit industry causing great economic losses. One of the most familiar parasitic diseases of rabbit is the hepatic coccidiosis which causes high mortalities in young rabbit's especially among the age between 4-8 weeks. Hepatic coccidiosis caused by *Eimeria stiedae* occurred in the liver which is the vital organ for protein synthesis and storage of most of nutritive body material. The prevalence of coccidiosis in young rabbits (weaning up to 2 months old) was 95 to 100%. Adult female rabbits usually acted as carriers within the farm and transmitted the parasite to young rabbits which caused severe infection with clinical sings and even death (Wang and Tsai, 1991).

Economic losses from coccidiosis which reaches 75.8% mortalities (Haiba *et al.*,1955), while (Arnoni 1978) showed that 48% of rabbit died on breeding farms of Pelotas, Weight loss and low food conversion as well as increased susceptibility to food after bacterial and viral disease.

Pasteurellosis remains a common disease in commercially produced rabbits (Digiacomo *et al.*, 1983). Attention should be drawn to the fact that Pasteurella multocida is normal inhabitant of the upper respiratory tract of rabbits (Hippe, 1982) and when animals are subjected to stress factors the organism may provoke the disease.

Pasteurella multocida vaccines were moderately successful in protection against pasteurellosis in rabbits (Okerman and Spanoghe, 1981).

So this study was carried out to investigate the effect of coccidial infectation on the immune response of rabbits vaccinated with oil adjuvant pasteurella multocida vaccine.

Materials and methods

Bacterial strains. Vaccinal *P. multocida* serotype A and D are routinely used in the production of inactivated oil adjuvant polyvalent

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rabbit pasteurellosis vaccine obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt

Isolation and sporulation of *Eimeria stiedae* oocysts. This step was carried out according to (Abd El- Rahman, 1988), where *Eimeria stiedae* sporulated oocysts was provided and characterized by Deptartment of Parasitology, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The obtained oocysts were washed with normal saline to remove the bile then transferred to a clean sterile Petri-dish containing 2.5% potassium dichromate solution for a depth of 3-5 mm and incubated at 26°C with a relative humidity 76-80%. Sporulation of the oocysts was followed up daily until complete sporulation was obtained, the contents of the Petri- dish were centrifuged at 3000 rpm for 1.5 minutes where the supernatant was discarded and the sediment was washed several times with distilled water and re-centrifuged as before until the supernatant become clear. The sediment was re-suspended in 2.5% potassium dichromate solution and stored at 40°C until used.

Propagation of *Eimeria stiedae*. It was carried out according to Zhang *et al.*, (1996) where 10 rabbits of one month old free from Eimeria were infected with 30000 freshly sporulated oocysts of *Eimeria stiedae*, rabbit inoculated per os (Ragab, 2001) then the oocysts were collected from the gall bladder of slaughtered rabbits 1week post infectation and sporulated as mentioned above.

Storage of sporulated oocysts. Sporulated *Eimeria stiedae* oocysts were stored in 2.5% potassium dichromate solution at 4°C as mentioned (Abu El-Ezz, 1994).

Anti coccidial drug. Aviax 5% (semdurmycin 150) was supplied by (Phibro) and used through mix of 500gm/ 1000kg of finished feed to provide 25 ppm of semduramycin up to 5 days.

Rabbits. A total of 60 New Zealand rabbits were used (4 to 8 weeks old and about 1.5 kg body weight). These rabbits were chosen from flocks with neither history of Pasteurellosis nor vaccinated against the *Pasteurella multocida*. No antibodies to *Pasteurella multocida* were detected in blood sample. Nasal swabs from the rabbits were cultured on blood agar and proved to be negative for *Pasteurella multocida*. Rabbits were grouped and vaccinated as shown in Table (1).

Samples. Fecal samples were collected and examined by using flotation technique from 5th day till the third week post infection. Also oocysts count was carried out using McMaster technique. Also blood sample were collected from the ear vein before vaccination, after the first and second dose of vaccination, then every two weeks till the end of experiment from each groups. Sera were separated and stored at -20°C till used.

Indirect heamagglutination test (IHA). It was done according to Carter and Rappay, (1962).

Enzyme linked immunosorbent assay (ELISA). It was carried out according to Briggs and Skeels, (1984).

Table (1): Experimental schedule.

Animal group	Subgroups	No of rabbits	Vaccinated group	Infested group	Treatment[1]	Challenge
A	A1	6	1 ml containing 10^9 CFU P. multocida S/C at 4 weeks (1st dose) then at 8 weeks (2nd dose	Infested at time of the 1st dose of vaccine	Treated	0.2 ml S/C x 10 LD50 diluted from a concentration of 10^9 CFU of *P. multocida* cell suspension after 2 weeks from 2nd dose of vaccine
	A2	6			Non	
B	B1	6		Infested after 15 days from 1st dose of vaccine	Treated	
	B2	6			Non	
C	C1	6		Infested at time of the 2nd dose of vaccine	Treated	
	C2	6			Non	
D	D1	6		Infested after 15 days from 2nd dose of vaccine	Treated	
	D2	6			Non	
E		6		Non	Non	
F		6	Non	Non	Non	

[1] Treatment with Aviax 5 % (semduramycin) after 2 days from infestation with *Eimeria stiedae*.

Results and Discussion

Hepatic coccidiosis of rabbits has been investigated by many authors. This parasitic infection remains one of the most important disease problems in young rabbits than the growing ones because the susceptibility to infection in young rabbits is very high. This picture might be attributed to lack of immunity among young individuals, while it was gradually established among the growing mates (Calnek *et al.*, 1997).

Many immunological studies were carried out in the control of coccidiosis, several points need excessive investigation particularly those

Table (2): Effect of E. stiedae infectation and treatment on immunized rabbits with oil adjuvant Pasteurella multocida vaccine (against type A) using indirect haemagglutination test.

Animal group	Subgroups	Pre-vaccination	Weeks Post 1st dose of Vaccination					
			2	4	6	8	10	12
A	A1	12	204	301	670	912	1609	1990
	A2	12	66	126	359	590	1059	1360
B	B1	11	289	326	663	905	1512	1995
	B2	11	295	118	345	560	998	1349
C	C1	10	284	463	675	922	1614	2016
	C2	10	287	460	450	658	1060	1412
D	D1	11	289	450	885	952	1649	1971
	D2	11	290	456	889	495	970	1340
E		12	294	464	891	1197	2018	2521
F (Control)		11	11	12	13	13	12	10

Group (A1): Infected with the 1st dose of vaccine and treated.
Group (A2): Infected with the 1st dose of vaccine but did not receive treatment.
Group (B1): Infected at 2 weeks after 1st dose of vaccine and treated.
Group (B2): Infected at 2 weeks after 1st dose of vaccine but did not receive treatment.
Group (C1): Infected at the time of 2nd dose of vaccine and treated.
Group (C2): Infected at the time of 2nd dose of vaccine but did not receive treatment.
Group (D1): Infected after 2 weeks from 2nd dose of vaccine and treated.
Group (D2): Infected after 2 weeks from 2nd dose of vaccine but did not receive treatment.
Group (E): Vaccinated rabbits.
Group (F): Non vaccinated, non infected and non treated (control).

concerned with immunity and vaccination (Abdel Rahman, 1988).

Rabbit pasteurellosis and coccidiosis represents two major problems facing rabbit industry and may lead to complete destruction of a rabbit farm. So, the present work investigates the effect of rabbit coccidiosis and its treatment on the immune response of rabbit vaccinated with inactivated oil adjuvant haemorrhagic septicaemia vaccine. In a trial to answer the question about the administration of such vaccine to rabbits at the time of vaccination when such rabbits found to be infected with *Eimeria stiedae* or received a specific treatment as semduramycin 150.

The experimental infection of rabbits with *Eimeria stiedae* (group A, B, C, D) revealed loss of appetite, diarrhoea and distension of abdomen which agree with that recorded by (Rosimin and Simoni, 1979). The daily faecal examination for *Eimeria stiedae* oocyst revealed gradual decrease in number of oocyst till complete disappearance after 9-11 days post infestation in groups (A1, B1, C1, D1) which treated with semduramycin, while faecal samples from groups (A2, B2, C2, D2) which did not received the drug treatment showed the presence of Eimeria oocysts from the 7th day post infestation in 100% of rabbits till the 3rd week post infestation.

The humoral immunity was estimated by indirect haemagglutination test and ELISA technique as shown in Tables (2, 3, 4, 5). Rabbits vaccinated and infected with oocysts of *Eimeria stiedae* either at the time or after 15 days from first or second vaccination (subgroups A1, B1, C1, D1) and treated with semduramycin 150 showed slight decrease of the antibody titre against P. multocida type A and D then elevated and reaching their peak at 12 weeks post the 1st dose of vaccination. The above results revealed that the semduramycin ionphoric coccidostat plays an important role for protection of infested rabbits against coccidia and improved their immune status preventing failure of vaccination even when given at the time of vaccination. These results agreed with that of El-Schemy *et al.*, (2009).

In subgroups (A2, B2, C2, D2) which were vaccinated and infected at the time or after 15 days from the first or second vaccination but not receive treatment showed sudden decrease of P. multocida antibody titres after infestation then increased slowly till the end of the experiment. These results agreed with Barriga and Anani (1979) who reported that E. stiedae destroys part of the liver of rabbits (which in turn disturb liver enzymes. Furthermore, serum protein and immunoglobulin were affected (Haiba and Geneidy, 1965, Awadalla and Hegazi, 1992).

Table (3): Effect of *E. stiedae* infectation and treatment on immunized rabbits with oil adjuvant *Pasteurella multocida* vaccine (against type D) using indirect haemagglutination test.

Animal groups	Subgroups	Pre-vaccination	Weeks Post 1st dose of Vaccination					
			2	4	6	8	10	12
A	A1	12	198	298	675	915	1596	1990
	A2	12	60	120	350	585	1042	1360
B	B1	11	288	330	649	898	1529	1995
	B2	11	280	113	336	544	991	1349
C	C1	10	289	455	662	910	1632	2016
	C2	10	275	461	446	645	1056	1412
D	D1	11	283	458	879	960	1540	1971
	D2	11	278	456	872	481	950	1340
E		12	285	458	879	1198	2005	2521
F (Control)		11	11	12	13	13	12	10

Group (A1): Infected with the 1st dose of vaccine and treated.
Group (A2): Infected with the 1st dose of vaccine but did not receive treatment.
Group (B1): Infected at 2 weeks after 1st dose of vaccine and treated.
Group (B2): Infected at 2 weeks after 1st dose of vaccine but did not receive treatment.
Group (C1): Infected at the time of 2nd dose of vaccine and treated.
Group (C2): Infected at the time of 2nd dose of vaccine but did not receive treatment.
Group (D1): Infected after 2 weeks from 2nd dose of vaccine and treated.
Group (D2): Infected after 2 weeks from 2nd dose of vaccine but did not receive treatment.
Group (E): Vaccinated rabbits.
Group (F): Non vaccinated, non infected and non treated (control).

Table (4): Effect of *E. stiedae* infection and treatment on immunized rabbits with oil adjuvant *Pasteurella multocida* vaccine (against type A) using ELISA.

Animal groups	Subgroups	Pre-vaccination	Weeks Post 1st dose Vaccination					
			2	4	6	8	10	12
A	A1	90	705	1095	2270	3055	5309	6905
	A2	90	218	393	1230	1890	3259	4790
B	B1	92	982	1070	2259	3060	5293	6900
	B2	91	995	381	1265	1960	3360	4758
C	C1	95	985	1563	2290	3040	5213	6930
	C2	97	987	1569	1516	2171	3642	4769
D	D1	93	885	1568	2998	3005	5305	6895
	D2	90	989	1578	3020	1680	3290	4689
E		89	998	1578	3029	4070	6861	8571
F (Control)		90	91	94	93	89	82	75

Group (A1): Infected with the 1st dose of vaccine and treated.
Group (A2): Infected with the 1st dose of vaccine but did not receive treatment.
Group (B1): Infected at 2 weeks after 1st dose of vaccine and treated.
Group (B2): Infected at 2 weeks after 1st dose of vaccine but did not receive treatment.
Group (C1): Infected at the time of 2nd dose of vaccine and treated.
Group (C2): Infected at the time of 2nd dose of vaccine but did not receive treatment.
Group (D1): Infected after 2 weeks from 2nd dose of vaccine and treated.
Group (D2): Infected after 2 weeks from 2nd dose of vaccine but did not receive treatment.
Group (E): Vaccinated rabbits.
Group (F): Non vaccinated, non infected and non treated (control).

Table (5): Effect of *E. stiedae* infection and treatment on immunized rabbits with oil adjuvant *Pasteurella multocida* vaccine (against type D) using ELISA test.

Animal group	Subgroups	Pre-vaccination	Weeks Post 1st dose of Vaccination					
			2	4	6	8	10	12
A	A1	90	695	1040	1996	3069	5314	6908
	A2	90	220	385	1195	1865	3240	4730
B	B1	92	970	1030	2270	3065	5283	6890
	B2	91	980	365	1240	1920	3348	4748
C	C1	95	975	1545	2287	3031	5275	6930
	C2	97	983	1564	1502	2165	3609	4839
D	D1	93	975	1575	2963	3009	5294	6899
	D2	89	973	1563	2997	1710	3260	4781
E		89	985	1569	3019	4074	6852	8560
F (Control)		90	91	94	93	89	82	75

Group (A1): Infected with the 1st dose of vaccine and treated.
Group (A2): Infected with the 1st dose of vaccine but did not receive treatment.
Group (B1): Infected at 2 weeks after 1st dose of vaccine and treated.
Group (B2): Infected at 2 weeks after 1st dose of vaccine but did not receive treatment
Group (C1): Infected at the time of 2nd dose of vaccine and treated.
Group (C2): Infected at the time of 2nd dose of vaccine but did not receive treatment
Group (D1): Infected after 2 weeks from 2nd dose of vaccine and treated.
Group (D2): Infected after 2 weeks from 2nd dose of vaccine but did not receive treatment.
Group (E): Vaccinated rabbits.
Group (F): Non vaccinated, non infected and non treated (control).

Table (6): Results of challenge test for evaluation of the protective efficacy of rabbit groups to *P. multocida*

Animal groups	Subgroups	No. of rabbit	No. of dead	No. of survived	Protection %
A	A1	6	1	5	83.4 %
	A2	6	3	3	50 %
B	B1	6	1	5	83.4 %
	B2	6	3	3	50 %
C	C1	6	1	5	83.4 %
	C2	6	4	2	33.4 %
D	D1	6	1	5	83.4 %
	D2	6	4	2	33.4 %
E		6	0	6	100 %
F (Control)		6	6	0	0 %

Group (A1): Infected with the 1st dose of vaccine and treated.
Group (A2): Infected with the 1st dose of vaccine but did not receive treatment.
Group (B1): Infected at 2 weeks after 1st dose of vaccine and treated.
Group (B2): Infected at 2 weeks after 1st dose of vaccine but did not receive treatment.
Group (C1): Infected at the time of 2nd dose of vaccine and treated.
Group (C2): Infected at the time of 2nd dose of vaccine but did not receive treatment.
Group (D1): Infected after 2 weeks from 2nd dose of vaccine and treated.
Group (D2): Infected after 2 weeks from 2nd dose of vaccine but did not receive treatment.
Group (E): Vaccinated rabbits.
Group (F): Non vaccinated, non infected and non treated (control).

The results of vaccinated rabbits (group E) with oil adjuvant haemorrhagic septicaemia vaccine revealed a progressive and steady rise of antibody titre starting from the first week and thereafter post vaccination. These results were supported by Gergis (1993) and Lu *et al.*, (1988). The obtained results in Table (6) revealed that challenge test of all rabbit groups with the virulent *P. multocida* gave a protective percentage of 83.4%, 50%, 100%, 0% in treated, untreated, vaccinated and unvaccinated rabbits respectively. While, the protective percents insubgroups (C2, D2) were 33.4%. These results agreed with Gamal *et al.*, (2006). This may be

due to challenge concurrency post two weeks or at the time of infestation. From the obtained results, it could be concluded that the important recommendation to rabbit owners to avoid coccidian infestation during vaccination programs, which act as a stress factor and failure of the vaccine.

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تأثير الإصابة بالكوكسيديا استيدى على استجابة الارانب المناعية للقاح التسمم الدموى الأرنبى الزيتى متعدد العترات

استهدفت هذه الدراسة محاولة لتقييم الدورالمحتمل حدوثه نتيجة عدوى الكوكسيديا ستيدى على الأرانب المحصنة بالتسمم الدموى الأرنبى الزيتى ، تم تقسيم ٦٠ أرنب نيوزيلاندى إلى ستة مجموعات (A-F). قسمت كل من الأربع مجموعات الأولى إلى قسمين متساويين ، المجموعة (A1-A2) تم تحصينها وعدواها بطفيل الكوكسيديا (إيميريا ستيدى) مع الجرعة الأولى من اللقاح ، المجموعة (B1, B2) تم عدواها بعد أسبوعين من الجرعة الأولى للقاح، المجموعة (C1, C2) تم عدواها مع الجرعة الثانية ، المجموعة (D1, D2) تم عدواها بعد أسبوعين من الجرعة الثانية للقاح أما المجموعة (E) حصنت بجرعتى اللقاح والمجموعة (F) ضابطة. وقد أظهرت النتائج أن العدوى وقت أو بعد اسبوعين من التحصين مع الجرعة الأولى أو الثانية مع العلاج (A1, B1, C1, D1) بالسمديورااميسين إنخفاض طفيف فى المستوى المناعى بمقارنة المجموعة الغير معالجة (A2, B2, C2, D2) عند القياس باختبار التلازن الدموى الغير مباشر والاليزا، أما المجموعة المحصنة بجرعتى اللقاح فقط (E) فقد أعطت أعلى معدل مناعى، وقد أكدت هذه النتائج باختبار التحدى (فى جميع المجموعات) بميكروب الباستيريلا ملتوسيدا الضارى حيث اعطت نسبة حماية ٨٣.٤٪ ، ٥٠٪ ، ١٠٠٪ ، صفر٪ فى المجموعة المعالجة، الغير معالجة والمحصنة والضابطة على التوالى، لكن نسبة الحماية فى (C2, D2) هى ٣٣.٤٪ نتيجة إجراء اختبار التحدى بعد أو فى نفس وقت العدوى، وقد أشارت هذه النتائج أهمية منع حدوث عدوى الكوكسيديا أثناء برنامج التحصين للحصول على أعلى مستوى مناعى وأعلى نسبة حماية للقاح التسمم الأرانبى الزيتى.