

EFFECT OF PRE-LAMBING VACCINATION WITH CLOSTRIDIAL VACCINE ON SOME BLOOD CONSTITUENTS IN SHEEP AND THEIR LAMBS

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Abstract

Of twenty-two pregnant ewes, twelve were vaccinated by polyvalent clostridial vaccine (*Cl. perfringens* B and D, *Cl. oedematiens* and *Cl. chauvoei*). Forty-five days before lambing, a dose of 3 ml was given followed by 2 ml booster dose three weeks later. The other ten ewes were left without vaccination as a control group. Blood samples were taken from the dams of both control and vaccinated groups at birth 0, 15, 30 and 45 days and also from their lambs at 15, 30 and 45 days old. A significant decrease of serum GPT, GOT, alkaline phosphatase, total bilirubin, urea, creatinine and uric acid in vaccinated ewes and their lambs was noticed. Serum total protein was significantly increased on day 30 after birth in vaccinated ewes. The increase was mainly in the prealbumin and globulin fractions percentages. In lambs albumin fraction and gamma globulin, percentages were significantly increased in comparison with vaccinated group.

INTRODUCTION

Clostridial infection is widely spread all over the world and affection of all species was recorded. Infection of sheep in Egypt is common, especially lamb dysentery and pulpy kidney caused by *Cl. perfringens* group, accounting for a considerable portion of losses encountered in sheep production. The lethargy is due to the toxins secreted in the intestinal tract causing its severe damage, while, the

absorbed toxins lead to parenchymatous degeneration of internal organs. The efforts have been applied to combat the disease by vaccination, yet, sometimes the specificity of the formed immune bodies could not overcome the invading agent. Vaccination with polyvalent clostridial vaccine (*Cl. Perfringens* B and D, *Cl. oedematiens* and *Cl. chauvoei*) prepared according to procedure of Gadalla *et al.* (1971) was initially performed during the last third of pregnancy in ewes, in order to provide immune bodies for neonatal lambs via clostrum.

The physiological changes that occur after vaccination have not been widely investigated. This study is an endeavour to elucidate serum biochemical changes in ewes and their lambs after vaccination with the local polyvalent vaccine under natural environmental conditions.

MATERIALS AND METHODS

This experiment was performed on 22 pregnant ewes. Twelve ewes were vaccinated by polyvalent clostridial vaccine (*Cl. Perfringens* B and D, *Cl. oedematiens* and *Cl. chauvoei*) 45 days before lambing. A dose of 3 ml was given followed by 2 ml booster dose after 3 weeks. The procedure adopted for the preparation of the vaccine was that of Gadalla *et al.* (1971). Ten ewes were left without vaccination as a control group. Blood samples were taken from dams at birth 0, 15, 30 and 45 days post lambing and also from their lambs at 15, 30 and 45 old in both vaccinated and control groups.

Blood serum samples were analysed for determination of liver and kidney efficiencies and protein and its fractionation. The methods used were: Glutamic pyruvic transaminase "GPT" and Glutamic oxaloacetic transaminase "GOT" (Reitman and Frankel 1957), Alkaline phosphatase (Kind and King 1954), Bilirubin (Jendrassik *et al.* 1938), creatinine (Brod and Sirota 1948), Urea (Fawcett and Scott 1960), Uric acid (Caraway 1963) and total protein (Weichselbaum 1946). Fractionation of serum protein was performed by disc polyacrylamide gel electrophoresis according to the technique described by Jovin *et al.* (1971).

The data were tabulated and analysed statistically according to Hill (1977).

RESULTS

Table 1 shows a significant decrease of serum GOT, alkaline phosphatase, total

bilirubin, creatinine, urea and uric acid at birth, and at 15,30 and 45 days post-lambing in ewes after clostridial vaccination. In addition, serum GPT was also decreased significantly at birth only.

The data presented in Table 2 show that serum levels of GPT, GOT, alkaline phosphatase, total bilirubin, creatinine, urea and uric acid were significantly decreased in lambs obtained from vaccinated ewes.

The results presented in Table 3 explained that serum total protein level in ewes was significantly increased on the day 30 after birth, in vaccinated group in comparison with controls. The effect occurred mainly in the prealbumin fraction and globulin fraction percentages (alpha 1,2, beta2b and gamma 2a,2b)

In lambs (Table 4), albumin fraction and gamma globulin fraction percentages (1-b and 2-b) were significantly increased in comparison with vaccinated group.

DISCUSSION

Serum transaminase enzymes levels (GPT and GOT) in vaccinated ewes showed significant drop in their activities as compared to normal levels. In parallel with this effect, alkaline phosphatase and total bilirubin were significantly lowered after vaccination, while, the levels in control groups were within the normal ranges. This disagreed with Remberton et al. (1971) who reported a slight rise in GOT activity in sheep vaccinated with highly potent *CL.novy* type "B" vaccine until 32 hours after vaccination, then, returned to normal level within 50 hours after inoculation. Also Awad et al. (1985) illustrated a significant rise in GPT and GOT activities 15 days after the first dose of vaccination, by bivalent *Cl.perfringens* types "B" and "D" vaccine which decreased to normal levels 30 days after the first injection.

The levels of bilirubin in both vaccinated and control groups were higher in lambs than in their dams (Table 2). El-Amrousi et al. (1965) previously found that serum bilirubin in healthy sheep was 0.67 mg/100 ml and was in a higher levels in lambs.

Serum creatinine levels in ewes and in their lambs (Tables 1 and 2) were significantly lowered after vaccination of ewes which are within normal levels. Blood et al. (1979) found that the normal serum creatinine level in sheep was 1.2-1.9 mg/100 ml. When the level increased over 2.0 mg/100 ml, it indicated severe kidney damage (Ranganatha 1980). The levels of serum urea and uric acid decreased significantly in ewes and their lambs after vaccination. Awad et al. (1985)

Table 1. Serum levels in control ewes and those vaccinated with polyvalent clostridial vaccine during post - lambing period.

Parameters		Days after lambing			
		At birth (0)	15	30	45
GPT (U/ml)	Control	20.60 ± 1.11 ***	9.25 ± 3.12	9.00 ± 1.63	10.00 ± 2.47
	Vaccinated	4.00 ± 0.30	6.00 ± 0.93	7.75 ± 0.69	8.00 ± 0.93
GGT (U/ml)	Control	84.20 ± 6.62 ***	91.50 ± 5.60 ***	81.33 ± 0.31 ***	79.40 ± 2.50 *
	Vaccinated	39.00 ± 0.30 ***	41.00 ± 8.01	37.00 ± 7.91	71.75 ± 2.00 *
A.phosphatase (u/100ml)	Control	5.36 ± 0.77	9.74 ± 0.81	7.91 ± 0.62	10.10 ± 0.34 *
	Vaccinated	4.21 ± 0.48 *	5.67 ± 0.57	8.99 ± 1.20	8.67 ± 0.43 *
T. bilirubin (mg/100ml)	Control	0.18 ± 0.03 ***	0.22 ± 0.04 ***	0.28 ± 0.03 ***	0.21 ± 0.05 *
	Vaccinated	0.05 ± 0.01 ***	0.10 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
Creatinine (mg/100 ml)	Control	1.55 ± 0.10	1.90 ± 0.16	1.26 ± 0.05	1.52 ± 0.10
	Vaccinated	0.40 ± 0.02	0.35 ± 0.04 **	0.27 ± 0.03	0.24 ± 0.01 **
Urea (mg/100 ml)	Control	46.15 ± 2.01 **	62.04 ± 1.43 ***	59.96 ± 0.68	53.06 ± 2.50 *
	Vaccinated	17.61 ± 2.15 **	56.84 ± 3.48	33.96 ± 3.27	42.70 ± 2.67
Uric acid (mg/100 ml)	Control	0.55 ± 0.09 *	1.07 ± 0.05	0.74 ± 0.01	0.55 ± 0.04 **
	Vaccinated	0.23 ± 0.02	0.26 ± 0.05	0.30 ± 0.04	0.18 ± 0.02

Mean ± SE No. of animals : control 10 and vaccinated 12.

*P < 0.05 ** P < 0.01 *** P < 0.001

Table 2 . Serum levels in control lambs and those obtained from vaccinated dams.

Parameters		lamb's age		
		15 days	30 days	45 days
GPT (u/ml)	Control	10.60 \pm 0.59	10.50 \pm 0.16	16.33 \pm 2.20
	Vaccinated	12.00 \pm 2.37	9.00 \pm 0.56	8.25 \pm 0.25*
GOT (u/ml)	Control	82.00 \pm 2.26	57.00 \pm 0.95	80.67 \pm 4.88
	Vaccinated	72.00 \pm 4.92	52.00 \pm 11.21	36.75 \pm 3.45**
A. Phosphatase (u/100 ml)	Control	30.96 \pm 0.13	37.64 \pm 0.02	14.05 \pm 0.28
	Vaccinated	32.47 \pm 3.84	14.89 \pm 2.16**	7.57 \pm 0.32**
T.bilirubin (mg/100 ml)	Control	0.11 \pm 0.02	0.44 \pm 0.002	0.56 \pm 0.04
	Vaccinated	0.39 \pm 0.04**	0.20 \pm 0.01	0.12 \pm 0.01**
Creatinine (mg/100 ml)	Control	1.29 \pm 0.03	1.45 \pm 0.02	1.52 \pm 0.09
	Vaccinated	0.40 \pm 0.03**	0.43 \pm 0.02**	0.43 \pm 0.02**
Urea (mg/100 ml)	Control	50.43 \pm 2.09	40.87 \pm 1.02	40.78 \pm 0.46
	Vaccinated	35.21 \pm 0.40**	28.47 \pm 2.83**	36.89 \pm 0.12**
Uric acid (mg/100 ml)	Control	0.78 \pm 0.03	0.49 \pm 0.01	0.45 \pm 0.01
	Vaccinated	0.37 \pm 0.02**	0.45 \pm 0.06*	0.13 \pm 0.02**

Mean \pm SE

No. of cases: in control 10 and vaccinated group 12.

* P < 0.05

** P < 0.01

Table 3 . Serum proteinogram in control ewes and those vaccinated with polyvalent clostridial vaccine during post-lambing period.

Parameters		Post-lambing period			
		At birth(0)	15 days	30 days	45 days
T. Protein (gm/100 ml)	Control	4.83±0.23	4.37±0.03	4.34±0.06	4.33±0.24
	Vaccinated	7.84±0.01***	8.20±0.19***	8.97±0.19***	9.35±0.20***
Pre-albumin (%)	Control	0.78±0.11	0.88±0.09	0.64±0.06	0.86±0.17
	Vaccinated	5.58±0.10***	4.08±0.28***	2.88±0.14***	3.27±0.61*
Albumin (%)	Control	23.13±1.03	21.45±0.59	25.13±1.16	20.13±0.42
	Vaccinated	17.04±1.44**	24.10±0.90*	19.79±0.44***	22.02±0.33**
Total	Control	76.16±1.10	77.71±0.54	74.24±1.17	81.03±1.11
	Vaccinated	69.92±0.92***	71.33±1.21*	77.32±0.60*	74.66±0.85***
Alpha ₁	Control	6.39±0.32	6.07±0.31	6.07±0.64	8.49±0.91
	Vaccinated	11.26±0.34***	10.16±1.29***	11.60±1.76**	8.14±1.40
Alpha ₂	Control	3.51±0.60	2.15±0.02	3.07±0.33	3.52±0.29
	Vaccinated	9.58±0.73***	8.56±0.71***	6.39±0.28***	7.34±0.60***
Alpha ₃	Control	16.31±0.53	15.08±0.52	12.28±1.17	11.81±0.47
	Vaccinated	5.51±0.69***	4.12±0.50***	6.51±0.33***	4.02±0.13***
B ₁ -	Control	5.45±0.36	4.81±0.49	5.35±0.40	8.49±1.05
	Vaccinated	6.82±0.59**	4.41±0.32	4.88±0.12	3.81±0.28***
B ₂ -a	Control	3.99±0.43	5.24±0.30	6.20±0.70	7.16±0.44
	Vaccinated	4.81±0.18	5.06±1.15	10.47±0.86***	5.76±0.81
B ₂ -b	Control	5.49±0.44	5.00±0.66	5.59±0.23	7.08±0.61
	Vaccinated	8.65±0.57***	8.17±0.69**	4.94±0.12*	10.48±0.69**
Gamma ₁ -a	Control	20.04±2.67	23.34±1.06	20.70±0.23	19.45±0.40
	Vaccinated	4.54±0.39***	5.99±0.52***	8.72±0.83***	5.01±0.33***

Table 3. Cont.

Parameters	Post-lambing period			
	At birth(0)	15 days	30 days	45 days
Gamma _{1-b}	Control	3.77±0.29	4.23±0.23	4.86±0.48
	Vaccinated	3.12±0.68	4.25±0.40	6.51±0.19
Gamma _{2-a}	Control	2.59±0.26	3.77±0.45	3.69±0.32
	Vaccinated	4.24±0.27	6.34±0.11	4.22±0.20
Gamma _{2-b}	Control	8.62±0.43	8.96±0.12	7.04±0.26
	Vaccinated	11.41±0.77	13.77±0.87	13.08±1.61

Mean ± SE

No. of animals: Control 10 and vaccinated 12.

* P < 0.05

** P < 0.01

*** P < 0.001

Table 4. Serum proteinogram in lambs from control and vaccinated dams.

Parameters	Lamb's age			
		15 days	30 days	45 days
T. protein (g./ 100 ml)	Control	3.74 ± 0.05	3.90 ± 0.07	3.86 ± 0.03
	Vaccinated	7.76 ± 0.30*	8.00 ± 0.19*	7.89 ± 0.24*
Pre-albumin (%)	Control	0.58 ± 0.08	0.38 ± 0.03	0.39 ± 0.03
	Vaccinated	0.93 ± 0.15	0.67 ± 0.18	0.44 ± 0.06*
Alubumin (%)	Control	23.15 ± 0.24	24.07 ± 0.46	23.14 ± 0.29
	Vaccinated	42.16 ± 1.14*	37.28 ± 1.90*	32.18 ± 0.61*
Total	Control	76.27 ± 0.25	75.55 ± 0.49	76.46 ± 0.26
	Vaccinated	56.91 ± 1.06***	62.04 ± 2.01***	67.37 ± 0.67***
Alpha	Control	12.59 ± 0.17	13.65 ± 0.16	10.67 ± 0.06
	Vaccinated	10.49 ± 1.19	10.89 ± 0.98*	11.95 ± 1.05
B ₁	Control	17.86 ± 0.46	14.88 ± 0.28	14.12 ± 0.33
	Vaccinated	15.54 ± 0.10	12.62 ± 1.56	10.05 ± 0.15***
B _{2-a}	Control	6.10 ± 0.42	5.32 ± 0.53	6.28 ± 0.70
	Vaccinated	2.79 ± 0.50***	3.51 ± 0.86	4.04 ± 0.43*
B _{2-b}	Control	6.82 ± 0.77	9.50 ± 0.70	8.38 ± 0.84
	Vaccinated	2.45 ± 0.22***	2.54 ± 0.46***	3.61 ± 0.21***
Gamma 1-a	Control	6.43 ± 0.24	5.41 ± 0.14	6.72 ± 0.30
	Vaccinated	2.46 ± 0.04***	3.81 ± 0.30 ***	4.16 ± 0.11 ***
Gamma 1-b	Control	14.10 ± 0.26	15.44 ± 0.04	16.27 ± 0.56
	Vaccinated	14.81 ± 0.34	17.79 ± 0.93*	18.94 ± 0.32***
Gamma 2-a	Control	4.42 ± 0.27	3.08 ± 0.09	5.67 ± 0.31
	Vaccinated	3.52 ± 0.21*	4.82 ± 0.48**	5.60 ± 0.45
Gamma 2-b	Control	2.66 ± 0.13	2.41 ± 0.02	3.10 ± 0.30
	Vaccinated	3.39 ± 0.45	4.05 ± 0.53**	5.68 ± 1.05*

Mean ± SE

No. of cases : in Control 10 and vaccinated group 12.

* P < 0.05

** P < 0.01

*** P < 0.001.

demonstrated that the use of bivalent *Cl. perfringens* (B and D) vaccine (with 2 doses of 3.0 ml followed by 2.0 ml 30 days later) did not produce any harmful effect on the kidney function in sheep.

From the fore-mentioned results, it is concluded that vaccination of dams with polyvalent clostridial vaccine did not show any rise of significant indication of hepatic and kidney dysfunctions in ewes and in their lambs.

The data in Tables 2 and 3 showed the distribution of the different serum protein fractions during post-lambing period in dams and their lambs. It also explored the effect of pre-lambing clostridial vaccination. Serum total protein in control ewes during the experimental period was slightly higher than the level registered in healthy ewes. Alpha globulin on the day of birth in ewes and beta globulin during the post-lambing experimental period in dams and their lambs were increased over the normal control levels. Serum gamma globulin in dams and in their lambs increased during the post-lambing period. After vaccination, total protein level increased with only significant difference at 30 days after birth in dams. Prealbumin and albumin fractions percentages increased significantly in dams and in their lambs than in unvaccinated group. On the other hand, total globulin percentage decreased significantly during the experimental period with significant rise of alpha_{1,2}, beta_{2b} and gamma_{2a, 2b} fractions in vaccinated dams. In lambs, the significant increase in globulin fractions occurred in gamma_{1-b, 2-b}.

The increase of these fractions described as immunoglobulins in vaccinated group, detected that maternal immunity has been verified under our natural environmental condition, and the transfer of natural antibodies to suckling lambs was not interfering. Kovalenko and Fedorov (1973) mentioned that vaccination of ewes one month before lambing with two doses fortnight apart with a bivalent vaccine against enterotoxaemia and Braxy gave the lambs satisfactory antitoxin titre ten days after ingesting colostrum, and were immune to challenge until one month of age. Awad et al. (1985) concluded that vaccination with bivalent *Cl. perfringens* (B and D) vaccine was followed by significant rise in serum total protein and globulin values 15 and 30 days after the first dose of vaccine. On the other hand, no clear pattern emerged in serum protein fractions in different groups of sheep and lambs experimentally induced enterotoxaemia by different procedures (Pathak and Parihar 1991). They concluded that the total proteins, albumin and globulin levels were not influenced by haemoconcentration due to diarrhea, and serous effusions into the peritoneal cavities, pericardial sacs and little pulmonary oedema.

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تأثير التحصين باللقاح الجامع للكلوستريديم قبل الولادة على مكونات الدم بالانغهام والحملاان

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تم أخذ عينات دم من إثنتى عشرة نعجة عشار تم تحصينها باللقاح الجامع (كلوستريديم برفرتجنس) نوع ب ، د و لكوستريديم أو ديمتيانس و كلوستريديم شوفياى) عند الولادة وايضا بعد ١٥ ، ٣٠ ، ٤٥ يوما من الولادة .

وفى نفس الوقت السابق تم أخذ عشره عينات دم من نعاى عشار غير محصنه استخدمت كضوابط للمعدلات الطبيعىه .

تم أخذت عينات دم من اثنى عشر حمل من نتاج النعاى السابق تحصينها وذلك فى أعمار ١٥ ، ٣٠ ، ٤٥ يوما . كما انه تم أخذ عينات دم من عشره حملاان نتاج النعاى الغير محصنه وذلك فى أعمار ١٥ ، ٣٠ ، ٤٥ يوما وقد استخدمت هذه المجموعه كضوابط للمعدلات الطبيعىه .

وقد استخدمت عينات المصل المأخوذه من عينات الدم السابقه لقياس نسبه كل من البروتين الكلى ، البيلروبين و الفوسفاتيز القلوى ، اوكسالاسيتك ترانسامينز ، جلوتاميك بيروفيك ترانسامينز وكذلك التحليل الكهربائى الجيلاتينى القرصى وذلك لدراسة التأثيرات المختلفه على وظائف الكبد والكليه ممثله فى أنزيمات الترانسامينز ، الفوسفاتيز القلوى، البيلروبين الكلى ، اليوريا ، الكرياتينين ، حمض اليوريك فى مصل انعاى المحصنه وحملاانها ، بينما زاد البروتين الكلى زياده معنويه فى مصل النعاى المحصنه بعد ٣٠ يوما من الولادة . وتعزى هذه الزيادة الى الجزء السابق للالبيومين وأجزاء الجلوبيولين . أما فى الحملاان فأن الزيادة المعنويه كانت فى تسبه الالبيومين وأجزاء الجاملوبولين وذلك مقارنه بالمجموعه المحصنه .