STUDIES ON THE EFFECT OF INFECTION AND VACCINATION WITH SHEEP POX VIRUS AND VACCINE ON THE IMMUNITY AND BLOOD CHEMISTRY OF TESTED SHEEP

By

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ABSTRACT

A total of 20 male lambs aged 4-6 months, were included in this study. They were proved to be clinically healthy and free from antibodies against sheep pox in their sera (seronegative). The animals were divided into four equal groups, the first and second groups were vaccinated with the field dose of sheep pox vaccine, while the other groups (Group 3 and 4) were left without vaccination. One month after vaccination, the 2nd (vaccinated) and the 3rd (non-vaccinated) groups were experimentally infected with the virulent sheep pox virus. All groups were kept under observation and strict isolation measures. Blood and serum samples were regularly collected from all the animals before and after inoculation for biochemical, immunological and haematological experiments. The results showed significant variations in biochemical constituents, immune response and haematological pictures between the different groups, which could help in field diagnosis.

INTRODUCTION

Pox virus diseases occur in most animal species and are of considerable economic importance. Sheep pox are still a cause of major losses specially in developing countries; sheep of all ages may be affected (**Singh and Srivastava, 1979; Jensen and Swift, 1982; Sewell and Brocklesby, 1990 and Ammar *et al.*, 1999**).

It is notifiable disease in most countries of the world (**Murphy *et al.*, 1999**).

Sporadic outbreaks still occur in Europe, for instance in Italy in 1983, Greece and Bulgaria both in 1995 and 1996 and Greece in 1997 and 1998 (**Kitching, 1999**).

Recently, in Egypt a large number of sheep farms were infected with sheep pox and most of the diseased animals were died (68.42%) (**Ammar *et al.*, 1999**).

A mass vaccination of all susceptible animals is the only mean for prevention and control of sheep pox disease (**Jadhav *et al.*, 1989**).

The purpose of the present study is to perform the antibody response, serum biochemical components and haematological alterations in vaccinated, infected, vaccinated then infected and non-vaccinated non infected sheep, that help in diagnosis and differential diagnosis between these conditions in the field.

MATERIAL AND METHODS

1. Animals:

Twenty, clinically normal, seronegative male lambs, 4-6 months old were employed in this study. They were of native breed and obtained from El-Wady El-Gedid governorate and were kept under observation for 15 days before being used. They were divided into four equal groups, the 1st and 2nd groups (GI and GII) were vaccinated with Kenyan sheep pox vaccine, each lamb received 0.5ml intradermally (I/D) (log_{10}^{3} $TCID_{50}$); the 3rd and 4th groups were kept as unvaccinated control. These animals were kept under hygienic conditions. One month post vaccination, the 2nd and 3rd groups were experimentally infected (challenged) through intradermal inoculation with the virulent sheep pox virus (Egyptian strain). Each lamb was inoculated with 0.5ml (log_{10}^{2} SID_{50}), in the ventral aspect of the tail. The challenged lambs were kept under observation and their rectal temperature and any sign or skin lesions were recorded daily for 3 weeks.

2. Viruses:

A. Sheep pox vaccine (Kenyan strain):

A tissue culture attenuated sheep pox vaccine was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI).

B. Virulent sheep pox virus (Egyptian strain):

It was also obtained from VSVRI. It was used in experimental infection of immunized (vaccinated) and susceptible lambs (GII and GIII).

3. Anti-ovine IgG peroxidase conjugate:

It was supplied by Sigma, USA and used in enzyme linked immunosorbent assay (ELISA).

4. Blood:

Blood samples were collected regularly from the jugular vein in sterile venoject tube. Total and differential leucocytes and platelet counts were done according to **Schalm *et al.*, (1975) and Dodds, (1989)**.

5. Serum samples:

All vaccinated, non-vaccinated and challenged (experimentally infected) lambs were bled before inoculation as well as at weekly intervals after vaccination and challenge for 8 weeks. The sera were examined for:

A. Humoral immune response:

It was carried out by:

1. Virus Neutralization Test (VNT):

According to **Martin *et al.*, (1975)** in which the collected sera were inactivated at 56°C for 30 minutes. VNT was performed in Vero cell cultures that obtained from Pox Dept. (VSVRI).

2. Enzyme linked immunosorbent assay (ELISA):

It was applied according to the method described by **House *et al.*, (1990)** on the serum samples previously collected from all lambs.

B. Biochemical assays:

For the determination of some biochemical parameters. Total serum protein levels were estimated according to the method described by **Hoffman and Richterrich, (1970)**, albumin and total globulins according to **Doumas *et al.*, (1971)**, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to **Reitman and Frankel, (1957)**. Alkaline phosphatase (AP) according to **Kilichling and Freiburg, (1951)**. Urea by **Tabacco, (1979)** and creatinine by **Husdan and Rapoport, (1968)**.

RESULTS

Clinical signs after vaccination and challenge:

Our results proved that there are no significant alterations from the normal clinical conditions after vaccination and no localized or generalized lesions observed. While, the post challenge reaction appeared on the control non-vaccinated lambs (GIII) on the 6th day in the form of rising body temperature which reached to 41.5°C and persisted for 5 days in addition to dermal eruption, accompanied with weakness, loss of appetite, difficult breathing. A circular hyperemic maculae which measures 3-5 cm in diameter appeared at the site of inoculation and become confluent and progressed into firm, flat circular papule and after 3 weeks it detached from the surface and undergoes necrosis. Eventually, a firm dry scab formed and detached leaving a scar (Photo 1 and Table 1).

DISCUSSION

Biochemical, immunological and haematological changes occurs in animals only not due to vaccination but also due to infection of vaccinated or non-vaccinated ones, which may interrupted with the judgement on the accuracy of vaccination or on the presence of infection. The aim of this study concerned the relation between the changes in these parameters in the former

conditions which will be help in the diagnosis and differential diagnosis in the field.

The clinical signs after vaccination and/or infection with sheep pox viruses (Table 1) predominated that the vaccinated lambs withstood the experimental infection with the virulent sheep pox virus, while the clinical signs were observed among the non vaccinated lambs which was in agreement with **Biberstein and Zee, (1990)** who reported that the immune status affect the severity of sheep pox and the recorded symptoms were as those described by **Martin, (1983) and Rizkallah, (1994).**

The haematological pictures differed in different groups. Table (2) indicated no significant alteration in the leucocytic count (8.93±0.43) in vaccinated lambs (GI), while a significant increase was recorded in lambs infected after vaccination (GII) (11.15±0.51). A severe increase was reported in the experimentally infected group (GIII) (12.12±0.46). The same changes nearly occurred in neutrophils % which increased to reach (52.71±3.12), (59.18±3.64) and (63.35±3.18) in GI, II and III, respectively. On the other hand, lymphopenia was clearly appeared in the infected group (GIII) other than (GII) while no significant change in vaccinated animals (GI) other than the negative control group (GIV).

A significant increase in monocytes and a decrease in eosinophils specially in GIII other than GII was reported. Our results were in agreement with those of **Plowright *et al.*, (1959) and Agag *et al.*, (1997)** and disagree with **Bhowmik *et al.*, (1986).**

Serological tests (SNT and ELISA reading) have been used for many years in abundant studies and have proved to be reliable. Table (3) showed that the neutralization indices (NI) varied from group to group, it was 1.5 in vaccinated animals (GI) and 2.2 in the animals infected after vaccination (GII) while it reached 2.6 in the infected animals (GIII) and not exceeded 0.5 (negative) in the non vaccinated control lambs (GIV). The antibody response after vaccination agreed with **Kalra *et al.*, (1982). Cottrall, (1978)** reported that NI ≥ 1.5 calculated as a positive immunity.

As shown in Table (4), the ELISA reading against sheep pox antigen, confirmed the results obtained by VNT, in which the infected lambs (GIII) have the highest reading (2.4), then the infected after vaccination (GII) (2.0), and the vaccinated lambs (GI) recorded (1.5), while negative results were shown in (GIV).

VNT and ELISA indicated that the antibody titres appeared by the 2nd week post vaccination or infection and increased gradually till reaching the maximum level one month post inoculation which agreed with those obtained by **Rizkallah, (1994) and Olfat, (2000).**

The serum biochemical findings were demonstrated in Table (5), which shows that the total serum protein in the different groups I, II and III were increased (hyperproteinemia) but the infected lambs recorded the highest

significant increasing other than GI (vaccinated) or GII (infected after vaccination) and they were 89.3, 83.6 and 75.4, respectively in comparison with GIV (control) 72.7, but the highest reading of the vaccinated animals reached 80.2 five weeks post vaccination which meaning slight difference from GII. The increasing of serum total protein was also observed by **Hafez and Agag, (1988) and Tawfik *et al.*, (1999).**

Decrease of albumin level was significantly recorded in the infected lambs (GIII) other than the other groups vaccinated (GI) or vaccinated then infected (GII). **Pratt, (1997)** reported that the albumin loss result from any diffuse liver disease which is clearly appeared in the infected animals (GIII). These results were in agreement with **Magda *et al.*, (1999) and Tawfik *et al.*, (1999).**

The significant increasing of total globulin especially in the infected animals (GIII) (65.8) and (GII) (57.6) is due to stimulation of the body defense mechanism to sheep pox antigen.

Reduction in the A/G ratio is the first indication of a protein abnormality and production of antibodies (**Yadav and Kalra, 1987**).

Table (6) show significant increase in serum ALT and AST among the infected groups II and III which could be attributed to impaired liver function which agreed with **El-Amrousi *et al.*, (1974); Martin, (1983); Agag *et al.*, (1997) and Magda *et al.*, (1999).** On the other hand, slight decrease of AP activity in the infected group (GIII) result from hyperthermal stress (**Wegner, 1973**).

Serum urea and creatinine levels are used to evaluate several function, so their significant increase in the infected groups (II and III) could be attributed to the impaired excretion and reduced glomerular filtration. Our results agreed with the finding reported by **Agag *et al.*, (1992) and Magda *et al.*, (1999).**

Serum biochemical examination performed that there are great disturbance in liver and kidney function in the infected groups other than the vaccinated or control ones.

Our results offer an explanation for some immunological, biochemical and haematological alterations which could be useful for differentiation between vaccinated, infected or infected after being vaccinated sheep with pox viruses in the field.

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Table (2): Total and differential leucocytic counts and platelets of different groups.

Group	Parameters	Weeks Post Vaccination and challenge								
		0	1	2	3	4 *	5	6	7	8
GI	Total leucocytic (10^3/mm^3)	7.91±0.29c	7.90±0.33 cb	8.31±0.37 cb	8.25±0.38cb	8.39±0.32 cb	8.45±0.39 cb	8.59±0.31b	8.81±0.25 cb	8.93±0.42 cb
	Neutrophils %	40.39±2.85cb	42.85±3.17cb	46.18±3.43cb	48.69±2.72b	49.16±2.86b	51.32±2.47b	52.97±3.16a	53.41±3.91a	52.71±3.12a
	Lymphocytes %	50.34±3.18c	48.83±2.39c	47.99±2.55b	44.83±3.14b	44.23±2.96cb	41.92±3.84a	41.15±2.73a	40.20±2.78a	40.16±3.11a
	Monocytes %	5.81±0.32c	5.7±0.41c	6.1±0.38b	6.0±0.27b	6.4±0.19a	6.1±0.31a	6.1±0.45a	5.9±0.39b	5.9±0.44b
	Eosinophil %	1.94±0.21c	2.73±0.54c	1.82±0.36b	1.51±0.29a	1.50±0.64a	1.53±0.28a	1.75±0.41a	1.82±0.59a	1.78±0.84a
	Platelets 10^3/µl	325	400	420	345	315	300	300	290	310
GII	Total leucocytic (10^3/mm^3)	8.10±0.26c	8.22±0.31c	7.95±0.33c	8.26±0.30c	8.42±0.29b	8.67±0.41b	9.34±0.59b	10.73±0.46 b	11.15±0.51 b
	Neutrophils %	41.46±3.72c	44.16±2.87cb	45.28±2.48cb	49.25±2.63a	51.94±2.81a	52.32±3.13a	55.80±2.61 b	58.11±3.18 b	59.18±3.64 b
	Lymphocytes %	50.12±2.86c	48.941±2.10	46.82±3.41b	43.16±2.80a	42.11±3.12a	41.28±3.34a	37.65±2.81b	35.71±3.15b	33.24±3.45a
	Monocytes %	6.19±0.92c	6.18±0.81b	6.44±0.21a	6.59±0.43a	6.28±0.35a	6.37±0.29a	6.58±0.45a	6.75±0.91a	6.82±0.36a
	Eosinophil %	2.35±0.71c	2.21±0.53a	1.82±0.32a	1.56±0.84a	1.49±0.66a	1.53±0.27a	1.69±0.47a	1.58±0.61a	1.55±0.38a
	Platelets 10^3/µl	320	400b	400	330	315	380	360	300	290
GIII	Total leucocytic (10^3/mm^3)	8.01±0.28c	No significant changes at this time (up to 4th week)				8.86±0.29b	9.92±0.34 a	11.94±0.55 a	12.12±0.46 a
	Neutrophils %	42.28±3.56c					47.83±5.71b	55.71±3.14ab	62.90±2.84b	63.35±3.18 a
	Lymphocytes %	50.12±2.86c					47.19±3.30b	41.11±2.91c	33.34±3.10b	30.15±2.85a
	Monocytes %	5.71±0.36c					6.09±0.27a	6.82±0.52a	7.27±0.46b	7.48±0.29b
	Eosinophil %	2.66±0.24c					2.81±0.33c	2.65±0.15c	1.74±0.24a	1.41±0.94a
	Platelets 10^3/µl	300					400	380	320	310
GIV	Total leucocytic (10^3/mm^3)	7.89±0.38 cb	No significant changes allover the experimental time							
	Neutrophils %	41.92±3.44c								
	Lymphocytes %	49.61±2.83c								
	Monocytes %	6.29±0.18c								
	Eosinophil %	2.74±0.45c								
	Platelets 10^3/µl	330								

- Data with same litters are non-significant with (F-test) at $P > 0.05$.
- Data with different litters are significant with (F-test) at $P > 0.05$.

GI : Vaccinated only with sheep pox vaccine.

GII : Vaccinated with sheep pox vaccine then challenged (infected) with the virulent sheep pox virus (VSPV).

GIII : Not vaccinated and infected with VSPV (Control infected group).

GIV : Control not vaccinated not infected.

* Challenge (Experimental infection) time.

Table (5): Total serum protein, albumin and globulin in the different groups

Time (Weeks)		Total protein (gm/l)				Albumin (gm/l)				Total globulin (gm/l)				A/G ratio			
		G I	G II	GIII	G IV	G I	G II	GIII	G IV	G I	G II	GIII	G IV	G I	G II	GIII	G IV
Post vaccination	0	73.2± 1.4a	71.7± 1.9a	72.4± 1.6a	72.6± 1.5a	27.3± 1.3a	27.1± 1.2a	26.6± 1.1a	28.7± 1.3a	45.7± 1.3a	44.6± 1.8a	45.8± 1.7a	43.9± 2.3a	0.59± 0.02a	0.60± 0.01a	0.58± 0.03a	0.65± 0.05a
	1	77.4± 1.6a	72.0± 1.5a	72.8± 1.5b	72.5± 1.7bc	27.1± 1.2a	27.3± 0.9a	26.7± 1.0a	28.5± 1.5a	50.2± 1.4a	45.0± 1.6cb	46.2± 1.2b	44.1± 1.4bd	0.54± 0.02a	0.60± 0.03a	0.57± 0.01a	0.64± 0.02a
	2	79.5± 1.8a	77.4± 1.3a	72.5± 1.8ab	71.9± 1.9bc	27.2± 1.0a	27.1± 1.0a	26.9± 1.2a	28.4± 1.9a	52.3± 1.1a	50.5± 1.2b	45.6± 1.7c	43.5± 1.6d	0.52± 0.04a	0.53± 0.02a	0.59± 0.01a	0.65± 0.02a
	3	79.8± 1.5a	79.5± 1.4a	72.6± 1.7b	72.0± 1.3c	26.9± 1.1a	26.6± 1.4a	27.0± 1.3a	28.8± 1.6a	53.0± 1.0a	53.1± 1.5bc	45.6± 1.4bc	43.2± 1.5bd	0.50± 0.05a	0.51± 0.01a	0.60± 0.03ab	0.66± 0.01ab
	4	80.1± 1.3a	80.2± 1.5a	72.5± 1.8bc	72.1± 1.4bc	26.8± 1.2cb	26.5± 0.7d	26.9± 1.2b	28.7± 1.3a	53.5± 1.4a	54.1± 1.8ab	45.6± 1.8c	43.5± 1.6bc	0.50± 0.02b	0.48± 0.04c	0.59± 0.02d	0.66± 0.02a
Post challenge	5	80.2± 1.2a	81.3± 1.2a	78.4± 1.5a	72.4± 1.8b	26.9± 1.3b	26.3± 1.2b	25.9± 1.4c	28.9± 1.3a	53.2± 1.7bc	55.2± 1.3a	52.5± 1.3bc	43.5± 1.2d	0.50± 0.03b	0.47± 0.02cb	0.49± 0.03d	0.66± 0.04a
	6	78.5± 1.9c	83.5± 1.3b	83.6± 1.2a	72.8± 1.3cd	26.6± 1.1ab	25.1± 1.3ab	24.6± 0.8cb	28.7± 1.5a	52.0± 1.5d	58.1± 1.6b	59.1± 1.2c	44.0± 1.7d	0.51± 0.03b	0.43± 0.04cb	0.41± 0.01dc	0.65± 0.04a
	7	75.3± 1.4c	83.9± 1.4b	88.4± 1.3a	72.7± 1.6cd	26.8± 1.7ab	25.5± 1.2ab	23.8± 1.0cb	28.6± 1.3a	48.5± 1.3c	58.3± 1.9b	64.5± 1.7a	44.2± 1.3d	0.55± 0.01b	0.43± 0.01cb	0.36± 0.02dc	0.64± 0.01a
	8	75.4± 1.2c	83.6± 1.5ab	89.3± 1.2a	72.7± 1.5cd	26.9± 1.1ab	25.8± 1.4ab	23.5± 1.3cb	28.5± 1.4a	48.5± 1.2c	57.6± 1.8b	65.8± 1.2a	44.3± 1.6d	0.55± 0.01b	0.44± 0.01cb	0.35± 0.01dc	0.64± 0.02a

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GIII : Not vaccinated and infected with VSPV (Control infected group).
GIV : Control not vaccinated not infected.
- Data with same litters are non-significant with (F-test) at $P > 0.05$.
- Data with different litters are significant with (F-test) at $P > 0.05$.

Mean ± SE: Mean ± Standard Error.

Table (1): Clinical response to vaccination and/or challenge with virulent sheep pox viruses in lambs.

Animal group	Number	Local reaction	Systemic reaction	Pyrexia	Protection %
GI	5	0/5	0/5	0/5	-
GII	5	0/5	0/5	1/5	100
GIII	5	5/5	5/5	1/5	0
GIV	5	0/5	0/5	0/5	-

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GIV: Control not vaccinated not infected.

Table (3): Neutralization indices of serum samples in different groups of lambs.

WPV⇨ / Group⇩	0	1	2	3	4*	5	6	7	8
G I	0.1	0.8	1.5	2.2	2.0	2.0	1.9	1.8	1.5
G II	0.0	0.7	1.4	2.0	2.1	1.9	2.3	2.3	2.2
G III	0.2	0.2	0.3	0.2	0.4	1.0	1.6	2.4	2.6
G IV	0.1	0.1	0.3	0.4	0.2	0.3	0.5	0.4	0.4

GI: Vaccinated only with sheep pox vaccine.
GII: Vaccinated with sheep pox vaccine then challenged (infected) with the virulent sheep pox virus (VSPV).
GIII: Not vaccinated and infected with VSPV (Control infected group).
GIV: Control not vaccinated not infected.
WPV: Weeks Post Vaccination.
NI ≥ 1.5 considered protective.
* Challenge (infection) time.

Table (4): ELISA readings of serum samples in different groups of lambs.

WPV ⇨ / Group ⇩	0	1	2	3	4*	5	6	7	8
G I	0.10	0.9	1.3	1.5	1.7	1.7	1.6	1.5	1.5
G II	0.15	1.1	1.5	1.8	1.8	1.6	2.0	2.0	2.0
G III	0.10	0.0	0.10	0.15	0.10	0.8	1.5	2.1	2.4
G IV	0.20	0.8	0.10	0.25	0.20	0.20	0.15	0.20	0.15

GI : Vaccinated only with sheep pox vaccine.
GII: Vaccinated with sheep pox vaccine then challenged (infected) with the virulent sheep pox virus (VSPV).
GIII: Not vaccinated and infected with VSPV (Control infected group).
GIV: Control not vaccinated not infected.
WPV: Weeks Post Vaccination.
* Challenge (infection) time.
N.B.ELISA reading ≥ 1.0 is considered positive.

Table (6): Liver function and kidney function in the different groups.

Parameters	G I (Mean ± SE)	G II (Mean ± SE)	G III (Mean ± SE)	G IV (Mean ± SE)
ALT (U/L)	30.28±0.64c	32.65±0.91b	36.18±0.62a	28.11±0.56d
AST (U/L)	31.22±0.41c	33.42±0.85b	38.19±0.83a	30.24±0.48d
AP (U/L)	7.32±0.46a	7.79±0.58ab	6.98±0.62ac	6.31±0.71d
Urea (mg/dl)	23.49±1.84cd	26.88±1.52b	31.67±1.43a	22.16±1.51cd
Creatinine (mg/L)	16.14±0.32cb	19.31±0.45b	23.46±0.19a	15.08±0.25d

- Data with same litters are non-significant with (F-test) at P > 0.05.
- Data with different litters are significant with (F-test) at P > 0.05.

ALT: Alanine aminotransferase.
AST: Aspartate aminotransferase.
AP: Alkaline Phosphatase.
SE: Standard Error.
GI: Vaccinated only with sheep pox vaccine.
GII: Vaccinated with sheep pox vaccine then challenged (infected) with the virulent sheep pox virus (VSPV).
GIII: Not vaccinated and infected with VSPV (Control infected group).
GIV: Control not vaccinated or not infected.
N.B. 1Serum samples were not frozen and stored at 4°C for not more than one week.
N.B.2 No significant changes in the different parameters were reported in vaccinated group (GI) allover the post vaccination time (For 8 weeks).

Photo (1): Lesion at the site of inoculation with the virulent sheep pox virus in control susceptible lambs

الملخص العربي

تأثير العدوى والتحصين بفيروس ولقاح جدري الأغنام على مناعة وكيمياء دم الأغنام المختبرة

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شمل هذا البحث على عدد عشرين حولي تتراوح أعمارهم من ٤-٦ شهور. وتم التأكد من أنهم في صحة جيدة ولا يحملون أجسام مناعية مضادة لجدري الأغنام في سيرم دمائهم. وقد تم تقسيمهم إلى أربع مجموعات متساوية حيث تم تحصين المجموعتين الأولى والثانية بالجرعة الحقلية من لقاح جدري الأغنام بينما تركت المجموعتين الثالثة والرابعة بدون تحصين، وبعد شهر من التحصين تم إجراء العدوى التجريبية (التحدي) للمجموعة الثانية (المحصنة) والمجموعة الثالثة (غير المحصنة). تم وضع كل المجموعات تحت الملاحظة وإجراءات العزل التامة. وقد جمعت بانتظام عينات الدم والسيرم قبل وبعد الحقن لإجراء التجارب البيوكيميائية، المناعية، الدموية عليها.

أظهرت النتائج اختلافات معنوية في المكونات ألبي وكيميائية والاستجابة المناعية وصورة الدم بين المجموعات المختلفة مما سيساعد في التشخيص الحقلي لها.