EFFECT OF SIMULTANEOUS VACCINATION WITH PASTEURELLA AND SHEEP POX ON THE BIOCHEMICAL COMPONENTS OF SHEEP BLOOD

By

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SUMMARY

Evaluation of the hematological, biochemical and immunological responses of sheep vaccinated with the inactivated ovine haemorrhagic septicaemia (HS) or sheep pox (SP) vaccine which given alone or simultaneously and/or experimentally infected with virulent sheep pox virus (VSPV) indicated that the hemogram picture showed a significant decrease of RBCs, Hb and PCV specially in the animals vaccinated with HS or those infected with VSPV. On the other hand, the leucogram picture indicated leucocytosis, neutrophilia, monocytosis and lymphopenia mainly in the lambs vaccinated or infected with SP viruses.

Total protein and globulin concentration increased in vaccinated and infected lambs and was accompanied by hypoalbuminaemia which lead to decrease of A/G ratio that meaning an abnormality of protein in addition to liver and kidney affection which also coincided with the results obtained from the testing of liver and kidney functions.

A significant decrease of thyroid hormones and an increase of cortisol specially after infection with VSPV was recognized as a result of stresses. The results proved that all groups of vaccinated lambs exhibited good levels of antibodies as measured against HS by the indirect haemagglutination test (IHA), mouse protection test and for SP by virus neutralization test (VNT) and for both microorganisms by ELISA test, in addition to a high rate of protection against the experimental infection.

The results indicated that there are no any antagonizing effect between the two antigens, and simultaneous vaccination of sheep with HS and SP vaccines protects them against infection with these diseases, which save time, stress and cost.

INTRODUCTION

Infectious disease continues to be one of the most important constraints on the efficient production of farm livestock in both developing and developed countries. While, vaccination and therapeutic or prophylactic use of drugs both play an important roles in animal disease control, vaccination is increasingly being viewed as the more sustainable option.

Pasteurellosis is to day one of the most serious diseases of livestock especially in the east, which responsible for respiratory disorders in sheep that remain a major cause of morbidity and mortality (Sutherland, 1985, sadiek et al., 1993 and Eman and Suzan, 2001). Pox virus diseases occur in most animal species and are of economic importance. Sheep pox is still a cause of major losses specially in developing countries (Sewell and Brocklesby, 1990, Fenner, 1996 and Ammar, et al., 1999).

In Egypt, the control of SP is successfully applied by tissue culture vaccine (Samir, 1994) and haemorrhagic septicaemia (HS) by formalized oil adjuvant vaccine (Geneidy et al., 1967).

Combined vaccines are of important approach to control different diseases. It is important to investigate how to use this combination without impairing the protection response to each of constituent antigens.

Previous reports indicated the validity of using haemorrhagic septicaemia vaccine simultaneously or in combination with other viral and bacterial vaccines (Kogan et al., 1966; Srivastava et al., 1976; Josef and Heidger, 1984; Rassmy, 1995 and Farid, 1996).

Sheep pox vaccine was found to be immunopotentiator when used with other vaccines (Taha et al., 1991; El-Shinnawy et al., 1993; Abeer, 1996 and Samir et al., 1999).

The aim of this study was to investigate the possibility of vaccinating sheep against both sheep pox and haemorrhagic septicaemia vaccines simultaneously and the effect of this vaccination on blood picture, hormones, some serum biochemical properties and immunological state of the animals.

MATERIALS AND METHODS

1. Animals:

Twenty-five Barki lambs, 6-8 months old, were obtained from El-Noubarya district and were kept under observation for 2 weeks before being used. The sera of these sheep proved to be free from sheep pox or pasteurella antibodies when examined serologically. They were divided into five equal groups as follows:

a. Group (1): Kept as a non-vaccinated non-infected control group.
b. Group (2): Non-vaccinated and then infected with virulent sheep pox virus.
c. Group (3): Vaccinated with the field dose (FD) of sheep pox vaccine (SPV) in the form of 0.5ml intradermally (I/D).
d. Group (4): Vaccinated with the (FD) of haemorrhagic septicaemia vaccine (HSV).
e. Group (5): Simultaneously vaccinated with both vaccines.

N.B. The last four groups were infected with VSPV one month post vaccination.

All of these lambs were kept under hygienic condition.

2. Blood and serum samples:

Two blood samples were collected aseptically from the jugular vein of the lambs before inoculation and at weekly intervals after vaccination and infection (for 8 weeks); the first sample was taken on EDTA for haematological studies and the second one was collected without anticoagulant for serum separation. The serum samples were freezed at -20°C until used for biochemical and immunological studies.

3. Vaccines:

a. Sheep pox vaccine:

Sheep pox vaccine (Kenyan strain) was kindly obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. It has a titre of $10^{5.5}$ $TCID_{50}$/ml (Samir, 1994).

b. Haemorrhagic septicaemia vaccine:

It was kindly obtained from VSVRI, Abbasia, Cairo. It was prepared according to Guneidy et al. (1967). It contained 3 X 10^9 viable cell/ml.

Animals of (G4 and G5) were inoculated S/C with two field doses of 1ml from H.S. vaccine, at one month apart, while the lambs of (G3 and G4) were vaccinated with 0.5 ml of S.P. vaccine intradermally (I/D).

4. Virulent sheep pox virus (VSPV) (Egyptian strain):

It was obtained from Pox Department, VSVRI, Abbasia, Cairo. It was used as a challenge virus for experimental infection of all lambs, one month post vaccination according to Sabban (1960).

5. Laboratory animals:

One hundred and five Swiss white mice 20 grams were used in passive mouse protection test. These were obtained from the Mice Farm, VSVRI, Abbasia, Cairo.

6. Haematological studies:

Blood was taken on EDTA for erythrocytic, total and differential leucocytic counts according to the standard techniques described by Schalm's et al. (1975) and Dodds (1989).

7. Biochemical assays:

Sera of different groups of sheep were examined for some biochemical parameters in which 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).

8. Hormonal assays:

Thyroid hormones i.e. thyroxine (T4) and triiodothyronine (T3); and cortisol hormone were estimated by Enzyme Immunoassay for quantitative measurement according to Buritis (1994). The used kits were (DSL-10-3200) for T4; (DSL-10-31005) for T3 and (DSL-10-2000) for cortisol in serum. Kits were purchased from Diagnostic System Laboratories, Inc. Corporate Headquarters, 445 Medical Center Blud. Webster, Texas 77598-4217, USA.

9. Immunological tests:

The sera were examined for estimation of antibody titre against sheep pox and/or H.S. by:

a. Virus neutralization test (VNT):

According to Martin et al. (1975) for evaluation of antibodies against SP.

b. Enzyme linked immunosorbent assay (ELISA):

According to the method described by House et al. (1990). This was used for all serum samples against both sheep pox and Pasteurella multocida antigens.

c. Indirect haemagglutination (HA) test:

It was carried out according to Carter and Rappy (1962) for detection of Pasteurella antibody titre.

10. Passive mouse protection test:

It was applied according to Alwis and Carter (1980). Sera collected from the vaccinated sheep with HS vaccine (G4 and G5) were tested for the presence of protective antibodies. Pooled sera from each group were injected into 5 mice subcutaneously (S/C) with 0.4ml/mouse; and challenged 24 hours post vaccination with 100 LD_{50} of virulent P. multocida. The mice were observed for one week.

The obtained data statistically analyzed according to Snedecor and Cochran (1990).

RESULTS

Clinical signs:

After experimental infection with VSPV, the vaccinated animals with SP vaccine (G3 and G5) gave no localized or generalized reaction, while the non-vaccinated lambs (G2) and the group vaccinated with HS vaccine alone (G4) showed severe local reaction accompanied with elevation of body temperature and appearance of different stages of pox infection.

DISCUSSION

Pasteurella is the etiological pathogen of different diseases among cattle, sheep, goat and other ruminants, exerting a considerable, world-wide economic impact on livestock industries (Gilmour and Gilmour, 1989 and Quinn et al., 2002). Pox infection also affects most animal species and causes considerable economic losses. Sheep of all ages may be affected with sheep pox (Singh and Srivastava, 1979, Sewell and Brocklesby, 1990 and Ammar et al., 1999).

New strategies are urgently required for development of new vaccine (Nagaraja et al., 1991). Protection of sheep against more

than one disease at same time is of great importance to reduce labor, costs and stress on vaccinated sheep.

Regarding to haematological results (Tables 1 and 2) changes occurred one week post vaccination in vaccinated groups (G3 and G4), it had high significant decreased levels of RBCs, Hb and PCV at $P < 0.02$; and high significant increased levels of MCV at $P < 0.05$ and MCH at $P < 0.02$. Three weeks post vaccination; RBCs and Hb of G3 were significantly decreased at $P < 0.05$ to (6.8 ± 0.1), (12.47 ± 0.29) respectively, accompanied with a significant increase of MCV at $P < 0.05$ to (44.98 ± 1.76) and MCHC (42.83 ± 0.48) (Table 1). Table (2) indicated that significant decrease in haemogram values appeared in G2 (infected with VSP virus) and G4 (vaccinated with HSV and then infected with VSPV).

These reductions in haematological parameter could be attributed to the inflammatory reaction (Bryson et al., 1979; Coles, 1986; Sadiek et al., 1993 and Khodary and Abdalla, 2001). The significant decrease of Hb % and PCV % values in sheep infected with sheep pox virus (G2 and G4) ranged between (11.05 ± 0.46 to 10.60 ± 0.15) for Hb % and (28.0 ± 0.92 to 29.0 ± 0.58) for PCV % which recorded in G2 four weeks post infection and in G4 early (after one week) in comparison with the control non-inoculated group (G1) (14.00 ± 0.37) of Hb % and (36.00 ± 0.33) of PCV %, this indicate severe anaemia which caused by infection. These results are supported by Radostits et al. (1995) and Zaghawa and Khalil (1997).

The leucogram picture of vaccinated and/or infected animals recorded in table (3 and 4) indicated no significant changes post vaccination except in (G4) which was vaccinated with (HSV) and showed a significant increase in total leucocytic count at $P < 0.05$ and neutrophil % at one and three weeks post vaccination and reached (9.2 ± 0.18 and 8.9 ± 0.13 X 10^3/cmm) and (42.30 ± 2.83 and 42.31 ± 1.32) respectively. Lymphopenia was also detected in which lymphocytes % decreased to (47.34 ± 1.23 and 46.40 ± 0.82) one and three weeks post vaccination.

The result obtained after challenge (experimental infection) with VSPV (Table 4) proved the occurrence of significant leucocytosis in (G2 and G3), which reached (12.02 ± 0.28) in the infected group (G2) and (9.96 ± 0.14) in group (3) which was vaccinated with SP vaccine before challenge with VSPV.

Monocytes % was also increased after infection and it reached (7.56±0.04, 7.10±0.02; 6.90±0.04 and 6.90±0.02) in G2, 3, 4 and 5 that appear in table (4) in comparison with the control non-infected group (G1) (5.8±0.02).

Table (5) indicates significant increase of total protein (hyperproteinemia), three weeks after vaccination at $P < 0.02$ which reached (8.90±0.2; 8.80±0.5 and 8.93±0.3) in G3, 4 and 5 in comparison with the non-vaccinated group (7.39±0.35).

In the infected group (Table 6) the total protein level reached 9.63±1.2 (G2) one month post infection in comparison with the control non-infected group G1 (7.79±0.2). These results was coincided with the results observed by Hafez and Agag (1988), Ashmawy et al. (1994) and Tawfik et al. (1999), who observed an elevation of serum total protein in some viral and parasitic infection. The albumin level recorded a mild decrease after vaccination but after experimental infection with VSPV (Table 6) a significant decrease was observed specially in G2 (infected animals) and G3 (previously vaccinated with SPV before infection) at $P < 0.02$. These results agree with Mottelib (1972) who explain that the decreased albumin level occurred due to the effect of bacteria and bacterial toxin on the liver cells producing impaired synthesis of serum albumin. These results were in agreement with Magda et al. (1999) and Manal et al. (2001).

On the contrary, total globulin level was significantly increased at $P < 0.02$ three weeks post vaccination from (4.76±0.09) to (6.28±1.00; 6.15±0.50 and 6.38±0.90) within the vaccinated groups (G3, G4 and G5) respectively (Table 5). After infection (Table 6), it recorded a highly significant increase, specially in the non-vaccinated infected group (G2) which was increased from (5.05±0.13) of G1 to (7.38±0.9) two weeks after infection. The significant increase of total serum globulin within the vaccinated or infected lambs may be due to stimulation of body defence mechanism to antigen of virus (Yadov and Kalra, 1987; Agag, 1997 and Magda et al., 2003). The increase of total globulin reflect on the level of total protein and this was in agreement with Alfonso et al. (1960); Desiderio et al. (1979) and Kaneko (1989). Yadov and Kalra (1987) and Agag (1997) reported that the increase of total serum globulins in the vaccinated or infected sheep may be attributed to the increasing of antibody production mechanism.

Table (5 and 6) indicated that the A/G ratio have been decreased three weeks post vaccination within the vaccinated groups (Table 5); and it was more prominent within the infected group (G2) and the group that vaccinated with SP before infection (G3) in which it was decreased from (0.56±0.08) to (0.32±0.01) and (0.37±0.07) two weeks post infection (Table 6). This decrease of A/G ratio is used to differentiate between different group which used as an indication of abnormality occur due to vaccination or infection and also for production of antibodies (Yadav and Kalra, 1987). The decrease of A/G ratio is also due to selective loss or decreased synthesis of albumin from chronic liver disease in which the liver is the sole site of albumin synthesis or due to increased globulins due to acute inflammatory disease, severe active hepatitis, acute nephritis, infectious diseases or dermatitis (Kaneko, 1989). Osbore and Vernier (1973) reported that albumin is selectively lost in renal disease.

Kaneko (1989) reported that the determination of serum protein and serum protein electrophoresis (SPE) profile have evolved into important diagnostic aids in clinical biochemistry and changes in A/G ratio are often the first signal of a protein abnormality.

Urea and creatinine are the smaller molecular weight compounds, while proteins represent one of the organic nitrogenous macromolecular compounds of the blood (Kaneko, 1989). Tables (7 and 8) shows that serum urea levels was significantly increased one week after vaccination in G4 (vaccinated with HSV) in which it elevated from (18.38±0.62) to (26.77±1.22) and also increased three weeks after infection specially in the sheep infected with VSPV (G2) which recorded (24.9±1.9) in comparison with the control group (G1) (16.35±1.30) which attributed to the impaired excretion and reduced glomerular filtration and kidney malfunction. Our results coincided with Agag et al. (1992) and Manal et al. (2001). On the other hand, the elevation of creatinine was less significant than that of urea.

Alkaline phosphatase (AP) level showed an increase at $P < 0.001$ one week post vaccination in (G4) that vaccinated with HS vaccine (Table 7). A significant increase of AP was reported in the infected group (G2) and in (G4) that infected after vaccination with HSV and reached after two and three weeks (20.0±0.95 and 21.69±1.82) and (20.15±22.35) respectively (Table 8). These results

agreed with Wegner (1973) and Manal et al. (2001) who reported that it may be due to hyperthermal stress.

Our result of ALT and AST value of (G4) vaccinated with HS vaccine showed a significant increase at $P < 0.02$ one week post vaccination (Table 8). One and two weeks after infection the ALT and AST levels of G2 and G4 exhibited significant increase and this elevation may be due to liver affection and general tissue damage caused by vaccination or infection. Agag et al. (1997) reported that the increase of ALT in infected sheep was occurred in association with pathological changes in liver. These results were in agreement with El-Amrousi et al. (1974); Agag et al. (1989) and Abou-Zaid et al. (1994).

Studying the effect of vaccination and/or infection on the thyroid hormones (Triiodothyronine "T3" and thyroxine "T4") and cortisol which affected by stress due to vaccination revealed the results recorded in Table (9 and 10) that reported decreasing of T3 and T4 one week post vaccination with SP vaccine at $P < 0.05$; a highly significant decrease was recorded in lambs vaccinated with HS vaccine at $P < 0.02$, which persist for three weeks post vaccination. After infection with VSPV the level of T3 and T4 showed a highly significant decrease at $P < 0.01$ in the infected group (G2) and in the group vaccinated with HS vaccine then infected with VSP virus (G4). These results agreed with Ganong (1979) and Kataria et al. (2000) who reported that the general stress depressed the secretion of thyroid stimulating hormone (TSH) through the depression of thyroid releasing hormone (TRH), therefore, thyroid hormone secretion decreases.

The mean value of T4/T3 ratio which show the activation of T3 transformation to T4 (El-Maghawry et al., 1993) increased according to stress of vaccination specially in sheep vaccinated with HS vaccine (Table 9). After infection with VSP virus (Table 10) the ratio still increasing after one and two weeks, and it recorded a high significant increase in the infected group (G2), two and three weeks post infection. These results were in agreement with Kataria et al. (2000).

The cortisol level showed a highly significant increase nearly parallel to the increasing of T3 and T4. These increasing level may be due to high level of stress on animal and this agree with James

(1992). Ninan and Vododria (2000) reported that the slight higher level of cortisol in ewes could be attributed to stress on animal.

Table (11) shows that the neutralizing indices (NI) against sheep pox differed according to the condition of different group; while it was reached 2.8 in infected group (G2), 2.5 in (G3) and 2.0 in (G5), it not surpass 0.6 in the control non-vaccinated non-infected sheep (G1). Cottral (1978) reported that NI > 1.5 meaning positive immunity.

ELISA reading against sheep pox (Table 12) revealed that it reached its highest level (2.20) in the infected group (G2), then in G3 it was (1.75) and in the animals of G5 it reached (1.52) and not surpass (0.28) in the control non-vaccinated, non-infected animals. These results affirmed the NI results and go along with Kalra et al. (1982).

ELISA results (Table 13) indicated that HS vaccine could protect the animals 25 days post vaccination and the antibody titre was more prominent in (G5) that simultaneously vaccinated with HS and SP vaccines. These serological results were in correspondent with biochemical and haematological results.

The indirect haemagglutination (IHA) test was applied to detect anti-HS antibody in livestock sera (cattle, buffalo, sheep and camel). Serum samples with a reciprocal haemagglutinating antibody titre less than 32 was considered negative (Quinn et al., 2002). The antibody titre reached 546.6 in (G5) and not exceeded 174.1 in (G4) in comparison with the control non-inoculated group (G1) in which its overall mean equal only 7.

In comparison between ELISA and IHA test, Scott et al. (1990) reported that ELISA detects not only agglutinating but also other subpopulations of immunoglobulin-G.

The results of passive mouse protection test (PMPT) (Table 15) showed that HS vaccine could protect mice against challenge with HS within 30 days post vaccination, while simultaneous vaccination with HS and SP vaccines gave earlier protection, 25 days post vaccination, that may be referred to the effect of sheep pox as an immunostimulator agent which is in full agreement with that described by El-Shinnawy (1993) and Abeer (1996). Chadrasekaran et al. (1994) reported that the PMPT has been

described as satisfactory for measuring immunity in vaccinated animals.

The results indicated that there are no any antagonizing effect between HS and SP antigens and simultaneous vaccination of sheep with HS and SP vaccines protects it against infection with these diseases without significant biochemical or hematological changes, which save time, stress and money.

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Table (1): Hemogram of lambs vaccinated with (SPV) and/or (HSV)

Time	1 Week Post Vaccination				3 Weeks Post Vaccination			
	G1+G2	G3	G4	G5	G1+G2	G3	G4	G5
RBCs X 10^6/cmm	8.05 ±0.32	7.95 ±0.19	6.03** ±0.17	6.58** ±0.21	8.166 ±0.11	7.88 ±0.21	6.80* ±0.10	8.11 ±0.10
Haemoglobin (g/dl)	14.83 ±0.33	13.34 ±0.49	11.82** ±0.58	12.85** ±0.45	13.23 ±0.95	13.09 ±0.33	12.47* ±0.29	12.90 ±0.34
PCV (%)	34.0 ±1.34	32.14 ±0.90	28.27** ±0.86	31.20 ±0.85	34.00 ±1.06	32.0 ±0.58	31.0 ±0.67	33.0 ±0.68
MCV (fl)	41.09 ±1.07	44.04 ±1.01	46.88* ±1.89	49.02* ±2.7	41.66 ±1.65	40.95 ±1.83	44.98* ±1.76	42.14 ±0.76
MCH (pg)	17.57 ±0.71	18.42 ±0.70	19.06* ±0.37	20.09** ±0.79	16.25 ±0.50	17.88 ±0.70	16.09 ±0.32	15.96 ±0.32
MCHC (g/dl)	43.96 ±0.91	41.84 ±0.51	42.0 ±1.09	39.93 ±0.99	34.28 ±0.82	41.14 ±1.28	42.83* ±0.48	37.11 ±1.47

* Significant at $P < 0.05$

** Significant at $P < 0.02$

G1 + G2: Control non-vaccinated group.

G3: Vaccinated group with sheep pox vaccine.

G4: Vaccinated group with haemorrhagic septicaemia vaccine.

G5: Group simultaneously vaccinated with both vaccines.

SPV: Sheep pox vaccine

HSV: Haemorrhagic septicaemia vaccine.

Table (2): Hemogram of lambs vaccinated with SPV and/or HS vaccine and then infected with VSPV

	One week post infection					Three weeks post infection					Four weeks post infection				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
RBCs ($X10^6$/cmm)	8.45 ±0.41	8.75 ±0.45	7.83 ±0.21	7.31 ±0.36	8.19 ±0.18	8.07 ±0.11	7.11 ±1.21	7.89 ±0.10	6.95* ±0.21	8.11 ±0.18	7.85 ±0.40	6.93 ±1.01	8.93 ±1.01	7.18 ±0.85	8.11 ±0.24
Haemoglobin (g/dl)	14.0 ±0.37	11.02** ±0.46	13.15 ±0.28	10.74 ±0.35	12.74 ±0.35	13.39 ±0.24	11.76* ±0.83	13.34 ±0.37	10.60** ±0.15	13.02 ±0.41	14.0 ±0.27	11.98* ±0.69	12.95 ±0.20	11.60** ±0.37	13.33 ±0.33
PCV (%)	35.0 ±0.68	31.0** ±1.71	34.0 ±0.60	29.00** ±0.58	36.0 ±0.42	36.0 ±0.33	30.02* ±0.45	34.0 ±0.42	30.0** ±0.79	36.0 ±0.85	35.0 ±0.35	28.0 ±0.92	36.0 ±0.60	32.0** ±0.48	34.0 ±0.80
MCV (fl)	41.35 ±0.54	36.85** ±0.71	39.93 ±0.82	37.04** ±0.92	41.16 ±1.79	44.06 ±0.51	40.39* ±1.09	43.09 ±0.95	40.55** ±0.15	43.98 ±0.70	44.08 ±0.81	40.46* ±0.62	44.92 ±0.98	42.93* ±0.61	44.65 ±2.25
MCH (Pg)	16.18 ±0.61	13.94* ±0.92	17.82 ±0.48	15.13* ±0.73	15.59 ±0.32	16.71 ±0.88	14.58* ±0.22	16.9 ±0.71	15.26 ±0.92	17.12 ±0.58	17.32 ±0.43	14.97* ±0.57	16.05 ±1.04	15.63* ±0.70	16.86 ±0.32
MCHC (g/dl)	40.0 ±1.31	34.69** ±0.58	38.06 ±0.77	35.19** ±0.79	38.75 ±1.38	37.01 ±0.89	42.93* ±1.32	37.4 ±1.11	40.5** ±1.32	38.99 ±0.57	40.0 ±0.52	33.81* ±0.94	38.64 ±0.89	35.37* ±1.24	39.11 ±1.47

* Significant at $P < 0.05$

** Significant at $P < 0.02$

G1: Control group non-vaccinated and non-infected (Control ve group).

G2: Infected group (Control +ve group) = infected with virulent SP virus.

G3: Vaccinated group with sheep pox vaccine then infected with virulent SP virus.

G4: Vaccinated group with HS vaccine then infected with virulent SP virus.

G5: Group simultaneously vaccinated with both vaccines then infected with virulent SP virus.

VSPV: Virulent sheep pox virus.

Table (3): Leucogram of lambs vaccinated with (SPV) and/or (HSV)

Time	1 Week Post Vaccination				3 Weeks Post Vaccination			
	G1+G2	G3	G4	G5	G1+G2	G3	G4	G5
WBCs X 10^3/cmm	7.61 ±0.11	7.70 ±0.17	9.20*** ±0.18	8.69 ±0.18	7.69 ±0.71	8.12 ±0.13	8.90*** ±0.13	8.01 ±0.18
Neutrophil (%)	35.30 ±0.94	39.21 ±1.71	42.3*** ±2.83	40.01** ±2.26	35.89 ±1.35	42.31*** ±1.32	44.21** ±2.58	40.85 ±1.39
Lymphocytes (%)	56.04 ±2.10	51.05 ±1.38	47.34*** ±1.23	50.05** ±1.92	55.58 ±3.05	48.13 ±2.13	46.40 ±0.82	50.09 ±1.64
Monocytes (%)	6.00 ±0.03	7.00 ±0.04	7.8 ±0.14	7.30 ±0.16	6.01 ±0.15	6.80 ±0.15	7.20 ±0.08	6.90 ±0.01
Eosinophils (%)	2.00 ±0.08	2.00 ±0.15	1.94 ±0.14	1.89 ±0.24	2.00 ±0.12	1.90 ±0.43	1.80 ±0.15	1.75 ±0.12
Basophils (%)	0.55 ±0.02	0.60 ±0.01	0.50 ±0.02	0.48 ±0.02	0.51 ±0.01	0.65 ±0.03	0.50 ±0.01	0.42 ±0.01

** Significant at $P < 0.05$
*** Significant at $P < 0.02$
G1 + G2: Control non-vaccinated group.
G3: Vaccinated group with sheep pox vaccine.
G4: Vaccinated group with haemorrhagic septicaemia vaccine.
G5: Group simultaneously vaccinated with both vaccines.
SPV: Sheep pox vaccine
HSV: Haemorrhagic septicaemia vaccine.

Table (4): Leucogram of lambs vaccinated with SPV and/or HS vaccine and then infected with VSPV

	One week post infection					Three weeks post infection					Four weeks post infection				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
WBCs ($X10^3$/cmm)	7.78 ±0.13	8.54 ±0.16	8.22 ±0.27	8.91 ±0.11	8.04 ±0.16	7.51 ±0.11	10.75** ±0.58	9.95** ±0.17	8.83 ±0.18	9.51** ±0.29	7.76 ±0.13	12.02** ±0.28	9.96 ±0.14	8.75 ±0.11	9.17 ±0.48
Neutrophils (%)	34.51 ±1.93	39.83 ±5.70	41.49 ±1.53	42.20 ±0.13	40.85 ±0.52	34.90 ±1.62	46.71** ±4.20	44.45** ±2.62	42.81 ±1.13	42.90 ±0.72	34.82 ±1.72	52.52** ±2.68	47.93* ±2.45	41.09 ±1.95	42.95 ±1.50
Lymphocytes (%)	57.32 ±2.64	51.26 ±3.34	49.36 ±3.45	48.90** ±2.18	50.10 ±2.18	56.51 ±1.07	44.30** ±5.90	46.53** ±1.94	48.00 ±1.16	47.60 ±1.89	56.92 ±1.72	38.55** ±2.68	43.13 ±0.45	49.65 ±0.95	47.75 ±0.50
Monocytes (%)	6.00 ±0.15	6.42 ±0.51	7.00 ±0.95	7.04 ±0.63	6.80 ±0.15	6.00 ±0.03	6.84** ±0.01	7.18** ±0.04	7.08 ±0.03	6.90 ±0.05	5.80 ±0.02	7.56** ±0.04	7.10 ±0.02	6.90* ±0.04	6.90 ±0.02
Eosinophils (%)	2.00 ±0.11	1.90 ±0.09	1.85 ±0.15	1.82 ±0.10	1.80 ±0.18	2.00 ±0.05	1.70 ±0.30	1.80 ±0.06	1.69 ±0.06	1.90 ±0.03	2.20 ±0.02	1.60 ±0.30	1.82 ±0.10	1.90 ±0.12	1.80 ±0.18
Basophils (%)	0.52 ±0.02	0.60 ±0.02	0.45 ±0.01	0.50 ±0.01	0.55 ±0.02	0.60 ±0.03	0.50 ±0.01	0.47 ±0.02	0.40 ±0.01	0.58 ±0.01	0.50 ±0.03	0.42 ±0.01	0.35 ±0.02	0.55 ±0.01	0.60 ±0.01

* Significant at P < 0.02

** Significant at P < 0.01

G1: Control group non-vaccinated and non-infected (Control -ve group).

G2: Infected group (Control +ve group) = infected with virulent SP virus.

G3: Vaccinated group with sheep pox vaccine then infected with virulent SP virus.

G4: Vaccinated group with HS vaccine then infected with virulent SP virus.

G5: Group simultaneously vaccinated with both vaccines then infected with virulent SP virus.

VSPV: Virulent sheep pox virus.

Table (5): Proteinogram of lambs sera after vaccination with (SPV) and/or (HSV)

Time	1 Week Post Vaccination				3 Weeks Post Vaccination			
	G1+G2	G3	G4	G5	G1+G2	G3	G4	G5
Total protein g/dl	7.25 ±0.50	7.85 ±0.90	7.92 ±1.10	7.70 ±0.90	7.39 ±0.35	8.89** ±0.20	8.80** ±0.50	8.93** ±0.30
Albumin g/dl	2.60 ±0.90	2.54 ±0.02	2.53 ±0.13	2.40 ±0.50	2.63 ±0.60	2.62 ±0.40	2.65 ±0.03	2.55 ±0.61
Globulin g/dl	4.65 ±0.15	5.31 ±0.60	5.39* ±0.90	5.30* ±0.70	4.76 ±0.09	6.28** ±1.00	6.15** ±0.50	6.38** ±0.90
A/G	0.56 ±0.04	0.48 ±0.04	0.47 ±0.09	0.45 ±0.03	0.55 ±0.05	0.42** ±0.05	0.34** ±0.07	0.40** ±0.02

* Significant at $P < 0.05$
** Significant at $P < 0.02$
G1 + G2: Control non-vaccinated group.
G3: Group vaccinated group with sheep pox vaccine.
G4: Group vaccinated group with haemorrhagic septicaemia vaccine.
G5: Simultaneously vaccinated with both vaccines.
SPV: Sheep pox vaccine
HSV: Haemorrhagic septicaemia vaccine.
N.B.: Nearly no variation was observed between the results obtained after 2 weeks and that found after one week and the same of the 3rd and 4th week post vaccination.

Table (6): Proteinogram of lambs sera after vaccination with SP and/or HS vaccine then infected with VSPV

	One week post infection					Three weeks post infection					Four weeks post infection				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Total protein (g/dl)	7.94 ±1.11	9.54* ±1.30	8.92* ±0.5	8.95* ±0.30	9.15* ±0.66	7.89 ±0.90	9.75** ±1.40	8.93* ±0.60	8.60* ±0.30	8.75* ±0.73	7.79 ±0.20	9.63** ±1.20	8.85* ±0.42	8.04* ±0.29	8.35* ±0.31
Albumin (g/dl)	2.81 ±0.29	2.63* ±0.70	2.76 ±0.30	2.82 ±0.60	2.75 ±0.09	2.84 ±0.13	2.37 ±0.90	2.41 ±0.06	2.73 ±0.07	2.68 ±0.03	2.79 ±0.125	2.42 ±0.20	2.49 ±0.10	2.65 ±0.30	2.60 ±0.50
Globulin (g/dl)	5.13 ±0.08	6.91* ±1.30	6.16* ±0.12	6.13* ±0.30	6.40* ±0.60	5.05 ±0.13	7.38* ±0.90	6.52* ±0.40	5.87* ±0.80	6.07* ±0.50	5.00 ±1.10	7.21* ±1.30	6.36* ±0.80	5.39* ±0.10	5.75 ±0.16
A/G	0.55 ±0.02	0.39* ±0.01	0.45* ±0.06	0.46* ±0.03	0.43 ±0.01	0.56 ±0.08	0.32** ±0.01	0.37* ±0.07	0.47 ±0.07	0.44 ±0.04	0.56 ±0.10	0.34** ±0.02	0.39* ±0.09	0.49 ±0.01	0.45 ±0.01

* Significant at $P < 0.02$

** Significant at $P < 0.01$

G1: Control group non-vaccinated and non-infected (Control -ve group).

G2: Infected group (Control +ve group) = infected with virulent SP virus.

G3: Vaccinated group with sheep pox vaccine then infected with virulent SP virus.

G4: Vaccinated group with HS vaccine then infected with virulent SP virus.

G5: Group simultaneously vaccinated with both vaccines then infected with virulent SP virus.

VSPV: Virulent sheep pox virus.

N.B. No significant variations was appeared one week post infection with VSPV.

Table (7): Results of liver and kidney function test in lambs vaccinated with (SPV) and/or (HSV)

Time	1 Week Post Vaccination				3 Weeks Post Vaccination			
	G1+G2	G3	G4	G5	G1+G2	G3	G4	G5
Urea mg/dl	18.38 ±0.62	21.10* ±0.52	26.77*** ±1.22	23.27 ±1.57	17.96 ±0.99	18.89 ±0.93	20.64 ±0.83	17.63 ±0.62
Creatinine mg/dl	1.43 ±0.05	1.53 ±0.07	1.645 ±0.04	1.59 ±0.06	1.38 ±0.07	1.43 ±0.07	1.51 ±0.06	1.48 ±0.05
A.P. u/l	16.41 ±0.28	18.50 ±0.62	19.30*** ±0.72	16.90 ±0.89	15.91 ±0.97	17.10 ±0.45	17.40 ±0.74	16.90 ±0.98
ALT u/l	26.50 ±0.44	28.20 ±1.50	30.70** ±1.20	28.90 ±0.76	26.95 ±0.58	27.30 ±1.02	27.05 ±0.30	27.00 ±0.26
AST u/l	28.20 ±0.58	28.93 ±0.26	29.40** ±0.96	28.83 ±0.66	28.60 ±0.40	28.90 ±0.19	29.30* ±0.25	28.46 ±1.50

* Significant at $P < 0.05$
** Significant at $P < 0.02$
*** Significant at $P < 0.001$
G1 + G2: Control non-vaccinated group
G3: Group vaccinated with sheep pox vaccine.
G4: Group vaccinated with haemorrhagic septicaemia vaccine.
G5: Simultaneously vaccinated with both vaccines.
SPV: Sheep pox vaccine
HSV: Haemorrhagic septicaemia vaccine.

Table (8): Results of kidney and liver function test in vaccinated and non-vaccinated lambs after infection with VSPV

	One week post infection					Three weeks post infection					Four weeks post infection				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Urea (mg/dl)	17.02 ±0.85	22.5*** ±1.05	19.65 ±0.88	20.11 ±0.70	19.01 ±0.15	16.35 ±1.30	24.9*** ±1.90	21.61 ±1.42	22.76 ±2.24	22.52*** ±3.58	17.68 ±1.34	25.00*** ±1.05	22.18 ±1.60	23.57*** ±1.60	21.34 ±2.27
Creatinine (mg/dl)	1.45 ±0.40	1.52 ±0.80	1.59 ±0.77	1.66 ±0.49	1.51 ±0.63	1.64 ±0.07	1.69 ±1.30	1.59 ±0.68	1.62 ±0.57	1.48 ±0.96	1.45 ±1.15	1.53 ±0.69	1.48 ±0.94	1.55 ±0.88	1.54 ±1.08
AP (u/l)	15.50 ±0.55	20.0*** ±0.95	19.57 ±1.13	20.15*** ±1.75	17.28 ±0.08	16.14 ±0.95	21.69*** ±1.82	17.10 ±1.13	22.35*** ±2.10	17.0 ±1.40	16.10 ±0.90	18.6 ±1.75	16.9 ±1.20	18.3 ±2.90	17.0 ±1.14
ALT (u/l)	28.10 ±1.12	32.5** ±1.56	30.5* ±1.84	31.05** ±1.88	29.74 ±1.40	27.90*** ±0.83	32.90*** ±0.92	30.10 ±0.75	30.30** ±1.05	28.36 ±1.45	28.04 ±1.22	31.92** ±1.50	29.90 ±1.46	30.19 ±1.20	28.59 ±1.11
AST (u/l)	30.50 ±0.64	37.12*** ±0.90	34.01* ±0.85	36.18*** ±0.62	31.65 ±0.41	29.85 ±0.55	36.20*** ±1.02	32.1* ±0.45	34.90** ±0.95	31.01 ±0.79	28.8 ±1.10	31.85** ±1.03	30.03 ±0.99	31.01** ±1.25	29.90 ±0.33

* Significant at P < 0.05
** Significant at P < 0.01
*** Significant at P < 0.001

G1: Control group non-vaccinated and non-infected (Control ve group).
G2: Infected group (Control +ve group) = infected with virulent SP virus.
G3: Vaccinated group with sheep pox vaccine then infected with virulent SP virus.
G4: Vaccinated group with HS vaccine then infected with virulent SP virus.
G5: Group simultaneously vaccinated with both vaccines then infected with virulent SP virus.
VSPV: Virulent sheep pox virus.

Table (9): Thyroid hormones and cortisol hormone in lambs sera after vaccination with (SPV) and/or (HSV)

Time	1 Week Post Vaccination				3 Weeks Post Vaccination			
	G1+G2	G3	G4	G5	G1+G2	G3	G4	G5
Thyroxine (T_4) ng/ml	145 ±17.50	136* ±12.50	115** ±12.80	130 ±13.59	155 ±13.97	150 ±14.06	134* ±14.03	145 ±11.99
Triiodothyronine (T3) ng/ml	2.75 ±1.90	2.30* ±2.00	1.80** ±0.95	2.00 ±2.10	2.90 ±1.20	2.45 ±0.99	1.84* ±0.23	2.37 ±0.05
T_4/T_3	54.95 ±7.50	57.43* ±5.30	63.50** ±5.04	59.54 ±6.00	56.47 ±4.16	60.50 ±6.50	65.85* ±7.11	61.08 ±9.05
Cortisol µg/dl	0.60 ±0.13	0.88** ±0.12	1.20** ±0.25	0.91 ±0.04	0.54 ±0.09	0.75 ±0.13	1.12** ±0.19	0.68 ±0.05

* Significant at $P < 0.05$
** Significant at $P < 0.01$
G1 + G2: Control non-vaccinated group.
G3: Group vaccinated with sheep pox vaccine.
G4: Group vaccinated with haemorrhagic septicaemia vaccine.
G5: Simultaneously vaccinated with both vaccines.
SPV: Sheep pox vaccine
HSV: Haemorrhagic septicaemia vaccine.

Table (10): Thyroid hormones and cortisol hormone in lambs sera after vaccination with SP and/or HS vaccine and then infected with VSP virus

	One week post infection					Three weeks post infection					Four weeks post infection				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Thyroxine (T_4) (ng/ml)	138 ±11.53	110*** ±13.59	120.4** ±12.5	115*** ±11.43	132 ±10.38	140 ±12.07	100*** ±14.38	132 ±10.61	115** ±11.05	142 ±15.89	140 ±13.40	117*** ±11.32	132 ±14.50	122** ±15.23	139 ±11.69
Triiodothyronine (T_3) (ng/ml)	2.90 ±0.52	1.792*** ±0.247	2.2* ±1.5	1.95*** ±0.225	2.40 ±0.21	2.75 ±0.443	1.28*** ±0.02	2.45 ±0.21	1.792** ±0.13	2.90 ±0.53	2.27 ±0.178	1.87*** ±0.198	2.30 ±0.09	2.0 ±0.266	2.25 ±0.222
T_4/T_3	55.87 ±5.9	69.60*** ±14.13	54.89 ±4.91	65.167*** ±1.85	52.5 ±1.59	51.99 ±5.42	75.2*** ±4.91	53.42 ±3.42	62.8** ±8.74	48.96 ±5.15	53.42 ±4.59	67.66*** ±10.16	54.0 ±5.03	53.42 ±4.73	55.18 ±7.34
Cortisol ug/l	0.62 ±0.03	1.20*** ±0.05	0.58 ±0.09	1.32*** ±0.13	0.58 ±0.04	0.58 ±0.07	1.43*** ±0.35	0.60 ±2.07	0.96** ±0.11	0.62 ±0.13	0.55 ±0.01	1.19** ±0.09	0.57 ±0.15	0.73 ±0.12	0.55 ±0.08

* Significant at $P < 0.05$

** Significant at $P < 0.02$

*** Significant at $P < 0.01$

G1: Control group non-vaccinated and non-infected (Control ve group).

G2: Infected group (Control +ve group) = infected with virulent SP virus.

G3: Vaccinated group with sheep pox vaccine then infected with virulent SP virus.

G4: Vaccinated group with HS vaccine then infected with virulent SP virus.

G5: Group simultaneously vaccinated with both vaccines then infected with virulent SP virus.

SP: Sheep Pox.

HS: Haemorrhagic Septicaemia.

VSP: Virulent sheep pox.

Table (11): Neutralizing indices of serum samples against sheep pox antigen

Animal groups / DPV	G1	G2	G3	G5
Zero	0.50	0.40	0.50	0.50
8	0.47	0.50	1.21	1.18
15	0.52	0.30	1.50	1.50
25	0.40	0.60	2.50	2.17
30 *	0.55	0.60	2.40	2.00
35	0.50	1.50	2.70	2.10
40	0.45	2.30	3.00	2.50
45	0.60	2.50	2.70	2.50
50	0.50	2.80	2.50	2.00
56	0.40	2.60	2.20	1.60

G1 & G2: Control non-vaccinated group.
G3: Sheep vaccinated with sheep pox vaccine.
G5: Sheep simultaneously vaccinated with sheep pox and haemorrhagic septicaemia vaccines.
DPV: Days Post Vaccination.
* Time of experimental infection with VSPV.
N.B. NI $\geq$ 1.5 is considered protective (Cottral, 1978).

Table (12): ELISA readings of serum samples against sheep pox antigen

Animal groups / DPV	G1	G2	G3	G5
Zero	0.25	0.20	0.25	0.15
8	0.24	0.25	1.02	0.90
15	0.18	0.20	1.82	0.53
25	0.22	0.15	1.80	1.53
30 *	0.25	0.80	1.85	1.60
35	0.28	1.40	1.75	1.65
40	0.21	1.80	1.85	1.70
45	0.22	2.00	1.93	1.60
50	0.20	2.10	1.75	1.52
56	0.24	2.20	1.75	0.90

G1: Control non-vaccinated group (-ve control).
G2: Non vaccinated and infected (+ve control).
G3: Sheep vaccinated with sheep pox vaccine.
G5: Sheep simultaneously vaccinated with sheep pox and haemorrhagic septicaemia vaccines.
DPV: Days Post Vaccination.
* Time of experimental infection with VSPV.
N.B. ELISA reading $\geq$ 1.0 is considered protective.

Table (13): Mean absorbance value in sera of sheep vaccinated with HS as measured by ELISA

Days Post Vaccination	Zero	8	15	25	30*	35	40	45	50	56	Overall mean
G1	0.003	0.008	0.004	0.003	0.008	0.006	0.007	0.005	0.003	0.004	0.005
G4	0.001	0.650	0.850	1.125	1.162	1.590	2.055	2.362	2.215	2.000	1.138
G5	0.003	0.785	0.940	1.312	1.571	1.902	2.470	2.420	2.516	1.560	1.548

N.B. ELISA reading ≥ 1.0 is considered protective.
* Second vaccinal dose.
G1: Control non-vaccinated group.
G4: Sheep vaccinated with haemorrhagic septicaemia vaccine.
G5: Sheep simultaneously vaccinated with sheep pox and haemorrhagic septicaemia vaccines.

Table (14): Anti-P. multocida titres in sera of differently vaccinated sheep as detected by indirect haemagglutination (IHA) test

Days Post Vaccination	Zero	8	15	25	30*	35	40	45	50	56	Overall mean
G1	6	7	6	8	9	7	5	8	8	6	7
G4	5	70	80	128	151	224	252	318	275	228	174.1
G5	8	137	233	462	575	693	814	916	925	711	546.6

* Second vaccinal dose.
G1: Control non-vaccinated group.
G4: Sheep vaccinated with haemorrhagic septicaemia vaccine.
G5: Sheep simultaneously vaccinated with sheep pox and haemorrhagic septicaemia vaccines.

Table (15): Passive mouse protection test (PMPT) in sera of sheep vaccinated with HS vaccine

Days post vaccination	No. of mice	HS oil adjuvant vaccine		No. of mice	Combined HS and sheep pox vaccine	
		Dead	Survived		Dead	Survived
0	5	5	0	5	5	0
8	5	5	0	5	4	1
15	5	4	1	5	4	1
25	5	2	3	5	1	4
30	5	1	4	5	0	5
35	5	0	5	5	0	5
40	5	0	5	5	0	5
45	5	0	5	5	0	5
50	5	0	5	5	0	5
56	5	0	5	5	0	5
Control	5	5	0			

الملخص العربى

تأثير التحصين المتزامن بالباستريلا وجدرى الأغنام على المكونات البيوكيميائية لدم الأغنام

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أوضحت نتائج تقييم الاستجابة الهيماتولوجية والبيوكيميائية والمناعية للأغنام الغير محصنة والمحصنة باللقاح المثبط للتسمم الدموى أو لقاح جدرى الأغنام التى أعطيت لها على حدة أو تزامنيا مع بعضهما والتى تم تجريبيا عدواها بفيروس جدرى الأغنام الضارى.

أوضحت النتائج أن صورة الدم أظهرت إنخفاضا معنويا فى كرات الدم الحمراء والهيموجلوبين وحجم الخلايا الحمراء خصوصا فى الحيوانات المحصنة بلقاح التسمم الدموى أو التى تم عدواها بفيروس جدرى الأغنام الضارى، وعلى النقيض فقد أوضحت النتائج زيادة عدد كرات الدم البيضاء، نسبة الخلايا المحببة (نتروفيل) والمونوسيت وإنخفاض نسبة الخلايا الليمفاوية خصوصا فى الأغنام المحصنة والمعدية بالجدرى. زاد البروتين الكلى وتركيز الجلوبيولين فى الحملان المحصنة والمعدية وكانت مصحوبة بنقص الألبيومين والذى أدى إلى إنخفاض نسبة الألبيومين/الجلوبيولين والتى تعنى إختلال فى البروتين بالإضافة إلى تأثر الكبد والكلى والذى تأكد من نتائج تجارب اختبار وظائف الكبد والكلى.

الإنخفاض المعنوى فى هرمونات الدرقية وزيادة الكورتيسول خصوصا فى المجموعة المعدية بالفيروس الضارى للجدرى تم تسجيله كنتيجة للإجهاد الناتج عن العدوى.

أثبتت النتائج أن كل مجموعات الأغنام المحصنة أظهرت مستوى جيد من الأجسام المناعية والتى تم قياسها للتسمم الدموى باختبارات تلازن الدم غير المباشر، حماية الفئران وللجدرى باختبار التعادل الفيروسى ولكل منهما باختبار الاليزا إضافة إلى المعدل المرتفع من الحماية ضد العدوى التجريبية (التحدى).

أوضحت النتائج البيوكيميائية والهيماتولوجية والمناعية أنه لا يوجد أى تأثير مضاد بين الأنتيجينين، وأن التحصين المتزامن للأغنام بلقاحى التسمم الدموى وجدرى الأغنام يحميها من العدوى بهذه الأمراض مما يوفر الوقت والجهد والمال.

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