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Evaluation of simultaneous vaccination against Brucella and Rift Valley Fever diseases in sheep.

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Brucellosis and Rift valley fever are major diseases affecting sheep flocks where they cause substantial economic losses in sheep and goats flocks. This study was planned to experimental evaluation of simultaneous vaccination against Brucella and attenuated RVF (smith burn strain) diseases in sheep using serological test including SNT and ELISA test revealed that there was no antagonizing effect between the two vaccines on the immune response of vaccinated animals and the results showed that no significance differences in antibodies titer in all sheep groups which vaccinated either with Rev-1 and attenuated RVF alone or simultaneously.

INTRODUCTION

Brucellosis and Rift valley fever (RVF) diseases have been recognized as the most important diseases affecting sheep in Egypt and all over the world causing sever losses in sheep population, resulting in sever economic losses.

Brucellosis is a bacterial disease caused by Brucella microorganisms that considered inter and intra-cellular parasites. Brucella is a worldwide problem for public health and animal production. Sheep brucellosis is a zoonotic disease caused by B. melitensis (biovar 1, 2 or 3), remains widespread worldwide (Gallein et al., 1998). B. melitensis has been recorded to be the main cause of brucellosis in Egypt in 1970 (Refai et al., 1990).

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Brucellosis affects many animal species as well as humans. Brucella abortus is the most important cause of bovine brucellosis, while both Brucella ovis and Brucella melitensis cause brucellosis in sheep (Timoney et al., 1988).

Vaccination represents an essential element in the control of bovine and ovine brucellosis. Live attenuated B. abortus 19 and B. melitensis Rev 1 have served as efficacious vaccinal strains for cattle and sheep, respectively (Blasco 1990 and Samartino et al., 2000).

The live Brucella melitensis Rev.1 strain is considered the best vaccine available for the prophylaxis of brucellosis of brucellosis in small ruminant caused by either B. melitensis or B. ovis. (Blasco 1996, El Idrissi et al., 2001 and Cloeckaert et al., 2004).

Rift valley fever (RVF) is an acute arthropod born viral disease affecting many species of animals like sheep, goats and cattle as well as human beings, characterizing by febrile illness, abortion of pregnant animals and high mortality rate among newly born animals (OIE 2000). It is an economically important viral disease and wildly distributed in different localities of Africa and Asia where periodic epizootic and epidemic accrued causing heavy losses among lambs and calves (Woods et al., 2002 and Fabgo 2002).

RVF disease appear for first time in Egypt during summer1977 in an epidemic form and reappeared again after 15 year as the 2nd epidemic in 1993 but it was in a milder form (El-Gabery et al., 1994) as well as (Who 2003) recorded 45 cases of RVF in August between Egyptian farmer in Kafer Al sheikh governorate

RVF disease is caused by RNA single stranded virus belonging to family Bunya viridae (WHO 1982 and Connie., 1996)

Controlling of the both diseases depend mainly on active immunization by vaccination of the animals using Brucella melitensis vaccine strain Rev.1 (Alton, 1985 and Fensterbank et al., 1982), and the attenuated RVF vaccine (Hassan 1998).

The current work aimed to test the possibility of using Brucella melitensis vaccine strain Rev-1 and attenuated RVF vaccine simultaneously and their effect on the immune response of sheep.

MATERIALS AND METHODS

Brucella references strains.

* Rev-1 (***B. melitensis*** biovar 1, vaccinal strain) was obtained from CVL, Weybridge, United Kingdom and used for preparation of ELISA antigen.
* S99 (***B. abortus***) Obtained from veterinary serum and vaccines research institute, Abassia, Cairo, Egypt, and used for preparation of Rose Bengal antigen.

Vaccines.

Two types of vaccines were used in this study. The Rev-1 vaccine (CZ Veterinaria S.A., Pontevedra, Spain), and Living attenuated Rift valley vaccine simth-burn strain (veterinary serum and vaccines research institute, Abassia, Cairo, Egypt).

Experimental design:

Animals

* Seventeen sheep 6 month of age were divided into four groups were as follows:

Group (1) 5 sheep injected s/c with Rev-1vaccine.

Group (2) 5 sheep injected s/c with living attenuated RVF vaccine.

Group (3) 5 sheep injected s/c with simultaneous Rev-1 and living attenuated RVF vaccines.

Group (4) 2 sheep were kept as control (non-vaccinated).

Vaccination with Brucella vaccine alone:

* Each animal in the corresponding group received a single S/C injection of 1 ml containing 2 × 109 CFU/dose (according to the manufacture instruction).

Vaccination with Rift valley fever vaccine alone:

* Each animal in the corresponding group received a single dose of 1 ml S/C (according to the manufacture instruction).

Sampling.

Blood samples were collected separately every week until the first month then after two week till the 3rd month, then monthly till 6th month. Serum samples were then collected by high speed centrifugation and kept in dry sterile capped tubes at –20°C till use.

Evaluation the immune response of the vaccinated sheep.

I-Rev-1 vaccine.

A- Rose Bengal test.

The collected serum was examined as described by ***Alton et al. (1988)***.

B-Indirect ELISA technique.

Serum samples were tested for the presence of anti- Brucella antibodies using ELISA test that utilize the whole cell antigen as described by Alton et al., 1988 and Colby et al., 2002.

II-Living attenatued Rift valley fever vaccine.

A-Serum neutralization test(SNT).

Were done according to *(Walker 1975).*

B-Indirect ELISA technique.

Were done according to *(Voller et al., 1976).*

RESULTS AND DISCUSSION

All serum samples collected from vaccinated sheep (either vaccinated alone or simultaneously with living attenuated RVFV is reacted positively with conventional Rose Bengal antigen.

Using the previously defined equation a negative cutoff value of each plate was calculated. Therefore, all serum samples (at 1/50 dilution) with a percent positivity ≥ cutoff value were classified as having a significant anti-brucella antibody response while samples with a percent positivity < cutoff value were considered no significant anti-brucella antibody response.

In case of vaccinated Sheep with Rev-1 vaccine alone (B. melitensis biovar 1), average value of these animals were positive or having a significant anti-brucella antibody response at 3, 6, 10, 12, and 16th weeks post-vaccination as shown in table (2&3).

It was noticed that all sera of vaccinated groups of sheep with RVF alone or simultaneous with Rev-1 vaccine detected antibody titers using SNT and ELISA, the protective level of the neutralizing index (NI) at 1st week post vaccination 1.6 of both (groups 2 and 3) where the acceptable limit of protection is 1.5 $\log_{10}$ $TCID_{50}$ as mentioned by ***(pini et al 1973)*** and reached the maximum peak to 3.8 at 12th week for both groups.

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Table (1): Results of antibody titers on sera of vaccinated sheep with Rev-1 Brucella vaccine alone or simultaneously with the RVF vaccine as tested by rough Rose Bengal test.

Weeks	Vaccinated animals	
	G1	G3
Pre vaccination	-	-
1st week	+	+
2nd week	++	++
3rd week	++++	++++
4th week	++++	++++
6th week	++++	+++
8th week	+++	++
10th week	+++	+++
12th week	++	++
16th week	+	+
20th week	+	+
24t Brucella references strains	+	+

* **G1**: Sheep vaccinated with Rev-1.
* **G3**: Sheep vaccinated simultaneously with Rev-1and RVF vaccine.

Table (2) Results of antibody titers on sera of vaccinated sheep with Rev-1 Brucella vaccine alone tested by indirect ELISA test.

Groups of animals	Optical Density											
	Weeks post vaccination											
	Pre vaccinati	1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week	16th week	20th week	24th week
(G1)	*0.010*	*18.47*	*43.38*	*46.58*	*52.29*	53.22	56.26	51.07	54.45	55.45	23.55	7.00

**G1: Sheep vaccinated with Rev-1.* **Cutoff value 46.58

Table (3) Results of antibody titers on sera of vaccinated sheep with Rev-1 Brucella vaccine alone or simultaneously with the RVF vaccine as tested by indirect ELISA test.

Groups of animals	Optical Density											
	Weeks post vaccination											
	Pre vaccination	1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week	16th week	20th week	24th week
(G3)	0.03	29.25	34.80	44.67	46.58	59.11	60.00	51.07	49.00	42.00	24.50	13.5

G3: Sheep vaccinated simultaneously with Rev-1 and RVF vaccines

**Cutoff value 38.7

Table (4): Result of Indirect ELISA technique of on sera of sheep vaccinated with *RVF vaccine alone or* simultaneously with the Brucella vaccine.

Groups of animals	Optical Density											
	Weeks post vaccination											
	Pre vaccination	1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week	16th week	20th week	24th week
(G3)	0.010	0.098	0.125	0.126	0.137	0.151	0.158	0.164	0.167	0.163	0.156	0.154
(G2)	0.012	0.091	0.124	0.128	0.134	0.144	0.153	0.157	0.165	0.162	0.158	0.153
(G4)	0.010	0.014	0.015	0.016	0.020	0.160	0.040	0.015	0.011	0.015	0.017	0.012

*Cut off value =0.03

G2: sheep vaccinated with Attenuated RVF vaccine.

G3: sheep vaccinated simultaneously with Rev-1 and attenuated RVF.

G4: sheep used as Negative control.

Table (5) Result of Neutralizing antibody index (NI) test on sera of vaccinated sheep with RFV vaccine alone or simultaneously with the Brucella vaccine.

Groups of animals	Neutralizing Indices											
	Weeks post vaccination											
	Pre vaccination	1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week	16th week	20th week	24th week
(G3)	0.3	1.6	2.1	2.2	2.7	2.9	3.2	3.6	3.8	3.7	3.4	3.2
(G2)	0.4	1.6	2.1	2.2	2.5	2.8	3.0	3.4	3.8	3.6	3.4	3.2
(G4)	0.3	0.2	0.2	0.2	0. 2	0.3	0.15	0.03	0.02	0.02	0.03	0.03

*G2: sheep vaccinated with Attenuated RVF vaccine.

*G3: sheep vaccinated simultaneously with Rev-1 and attenuated RVF.

*G4: sheep used as Negative control.

DISCUSSION

The simultaneous vaccinations has the advantage of providing protection against more than one disease at the same time, thus reducing vaccination expense and the number of vaccination as well as saving time and reduce stress reaction on the animals.

The control of Brucella and Rift Valley fever diseases are conducted by vaccination with specific vaccines which conferred a good degree of immunity against each one.

In the current work, a simultaneous vaccination with attenuated RVF vaccine and Brucella vaccine was performed aiming to study the effect of vaccination with the two vaccines on the immune response of sheep.

The Sera of sheep vaccinated with Rev-1 vaccine either alone or simultaneously reacted positively with conventional Rose Bengal from 1st

week post vaccination (+) and this reaction increased in strength till reach to maximum at 3rd to 8th week post vaccination (++++) and then decline to reach to minimum (+) at 18th week post vaccination (table 2).The humoral immune response of vaccinated sheep also measured by ELISA test which become a practical method for predicting the immunological response, the highest antibody level were recorded at 8th week post vaccination at cutoff value 46.58 and 38.7 respectively reaching 56.26 (group1) and 60.0 (group3).The previous results revealed that there is no significant difference in the antibody titers in sera of vaccinated sheep groups either alone with brucella vaccine or attenuated RVF vaccine or simultaneously with both vaccines (table 3 and table 4) that agree with (Gergis et al.,1992) who tested simultaneous vaccinated trail against Fowl Cholera ,New Castle and Fowl pox diseases) and disagree with (Afaf 1998) who reported that there is significant difference in groups of chicken vaccinated either alone with P .multocida living attenuated or Newcastle disease virus vaccine or combined with both vaccines and used combined vaccination gave high significant result than other vaccines, due to both living vaccines propagate in the host body produce large numbers of immunogenic antibodies and enhancement the humoral and cellular immune response, and the rout of administration are different,

and administrated initiated a direct and immediate influence on the immune system with a triple impact. The immune response of vaccinated sheep by attenuated RVF vaccine were tested by SNT, showed there is no significant difference in the neutralization indices in sera of sheep groups vaccinated either with attenuated RVF vaccine alone or simultaneously with brucella vaccine (table 5).The result of SNT was correlated with that obtained by ELISA test as shown in table (4&5) this results agree with (HASSAN 1998) and (Zeidan et al 2004) studied the immune response of pregnant cows to simultaneous vaccination with RVF and Entero-3 vaccines, they found the vaccines showed no antagonism between both of them.

(Naglaa et al 2005) studies the effect of simultaneous vaccination of cattle with Rift Valley Fever (RVF) or Bovine Ephemeral Fever (BEF) and Rabies vaccines on their immune response, revealed that there was no antagonized effect of the three vaccines on the immune response of vaccinated animals.

Also these agreement with (Hassan and Elmeneisy ,2006) who tested simultaneously vaccination of RVF inactivated vaccine and polyvalent clostridial vaccine in sheep and they showed that non-significant difference were noted between the serological tests results of the single and the simultaneously vaccination .

In this study revealed that absence of any side effect or symptoms of illness in all vaccinated sheep groups either alone or simultaneously with both vaccines during this study

It can be concluded that simultaneously vaccination of sheep save time, costs and efforts in combating such sheep disease.

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