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Brucella hot saline extract as an immune-stimulant agent with RVF vaccine in sheep

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SUMMARY

In this study three groups of sheep were used to evaluate the immunostimulant effect of Brucella hot saline extract (HSE) on the immunoresponses of sheep to inactivated Rift Valley Fever (R.V.F.) vaccine. 1ST group of sheep was vaccinated S/C with RVF vaccine alone and 2nd group of sheep was vaccinated S/C with RVF vaccine with Brucella HSE and 3rd group was kept as control. Serum samples were collected and examined using SNT which reveal that the immune-responses of sheep to RVF in group inoculated with *Brucella* HSE was more satisfactory with respect to RVF vaccine group and control group. Corresponding groups were done in mice for toxicity and ED50 test which gave results parallel to the SNT done on sheep serum. ED50/ ml in RVF vaccine with Brucella HSE and RVF vaccine alone were 0.003 and 0.002, respectively. While in SNT, Sheep vaccinated with inactivated RVF vaccine in combination with Brucella HSE gave high titers of antibodies from the 3rd week post-vaccination with a titer of 1.95 and reached its peak at 16th week post-vaccination (3.45) while sheep vaccinated with R.V.F. vaccine alone gave protective titer at the 3rd week post-vaccination (1.2) and reached peak of the titer at 14th week post-vaccination (2.85). Also immune responses of sheep to Brucella HSE were examined using Rose Bengal test which lasts for 24 weeks post-vaccination which mean that it can give protection against brucellosis which proved by measuring the protective activity of Brucella HSE in Balb/C mice which gave satisfactory results with respect of brucella vaccine and unvaccinated group.

Rift Valley fever (RVF) is a peracute or acute zoonotic, arthropod–borne viral disease of domestic ruminants. It is an economically important viral disease and widely 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).

Rift Valley fever (RVF) disease is caused by a RNA single stranded of a mosquito-borne bunyavirus of the genus Phlebovirus (WHO, 1982 and Connie, 1996). The disease occurs in climatic conditions favoring the breeding of mosquito vectors and is characterized by liver damage. The disease is most severe in sheep, goats and cattle, in which it produces abortions in pregnant animals and a high mortality rate in the newborn. Older non-pregnant animals, although susceptible to infection, are more resistant to clinical disease. There is considerable variation in the susceptibility to RVF of animals of different breeds. Camels suffer an unapparent infection with RVF, but abortion rates can be as high as in cattle. Humans are susceptible to infection through contact with infected material or mosquito bites (OIE, 2010).

RVF disease appeared for first time in Egypt during summer 1977 in an epidemic form (Imam et al., 1977) and reoccured after 15 years latter as the 2nd epidemic in 1993 but it was in milder form (El-Gabery et al., 1994) as well as (WHO, 2003) recorded 45 cases of R. V. F. in August between Egyptian farmer in Kafer Al-Sheikh Governorate.

Controlling of RVF depends mainly on active immunization by vaccination using A live vaccine prepared from Smith-burn's attenuated strain of RVF virus has been used for the control of RVF in non pregnant cattle and sheep in endemic areas and during outbreaks while inactivated vaccines is used in pregnant animals and in RVF-free countries are prepared from virulent field strains (Hassan, (1998) and Botros et al., (2006).

Chosen of Gram negative bacteria (as hot saline extract of *brucella* and flagelline of *E.coli*) especially proteins of cell wall of Gram negative bacteria as immunostimulant is a trend that had been explained by many reviewer as Petrunov et al., (2007) who explained that Gram-negative bacteria contain LPS, endotoxins, peptidoglycans and lipoproteins which stimulate macrophages, NK- cells, B-lymphocytes and antibody production and release of α- and γ- interferons and IL-2, IL-6. Also, Lipopolysaccharide (LPS) is a component of the outer membrane of Gram negative bacteria, It is generally considered to be the most potent immunostimulant among cell wall components, and consists of polysaccharide extending outward from the cell surface and a Lipid portion which is embedded in the membrane. This portion is known as Lipid A and is responsible for provoking immunostimulatory responses such as

production of pro-inflammatory cytokines and inflammatory effectors substances such as nitric oxide (Kaisho and Akira, 2002; Akira and Hemmi, 2003). In addition to the high concentrations of LPS generally required to induce immune responses, it seems that other pattern-recognition receptors (PRRs) such as beta 62 integrins may play a vital role in LPS recognition by piscine immunity (MacKenzie et al., 2003; Iliev et al., 2005).

The current work aimed to test the possibility of using Brucella HSE to increase the immune responses against the RVF vaccine.

MATERIALS AND METHODS

Brucella strain:

Brucella abortus S19 (CZ Veterinaria S.A., Pontevedra, Spain) used for preparation of Brucella Hot Saline Extract (HSE) according to Plackett et al., (1976).

Brucella melitensis biovar 3 (field isolate) used for challenge.

Inactivated RVF vaccine:

Inactivated RVF vaccine was prepared in (RVF department. VSVRI, Abassaia) according to Eman, (1995).

Preparation of RVF vaccine with Brucella HSE:

Inactivated RVF Vaccine was prepared and mixed with Brucella HSE with a final concentration 8 mg HSE/ dose.

Animals:

Mice:

Adult Swiss albino mice were used for toxicity and potency tests for the prepared RVF vaccines.

Adult female Balb/C mice used for evaluation of the protective activity of Brucella HSE against Brucella.

Sheep:

Six lambs of 5-10 days old were used for evaluation of the safety of the prepared RVF vaccines

Ten sheep of 6 months of age free from antibodies against RVF and Brucella were used for evaluation of prepared vaccines.

Experimental design:

Ten sheep were divided into 3 groups:

Group (1) 4 sheep injected S/C with 1 ml of inactivated RVF vaccine/ sheep contain 8 mg of Brucella HSE.

Group (2) 4 sheep injected S/C with 1 ml of inactivated RVF vaccine/ sheep

Group (3) 2 sheep were kept as control (non-vaccinated)

Evaluation of the prepared vaccines:

Sterility test: was done according to OIE, (2010).

Safety test: was done in both lamb and Swiss albino mice according to Eman, (1995).

1. **Potency test:** were done according to Randall et al., 1964 using adult Swiss albino mice.

Sampling:

Blood samples were collected separately every week for two months then every two weeks for 4 months till the end of 6th month post-vaccination. Serum samples were then collected by high speed centrifugation (1500 rpm/15 min) and kept in dry sterile capped tubes at -20C till use.

***Evaluation of the immune-responses against inactivated RVF vaccine:**

1. Serum neutralization test was done according to (Walker, 1975)

2. Effective dose that protect 50% of mice (ED50) was done according to (OIE, 2008).

***Evaluation of the immune-responses against Brucella HSE:**

1. Rose Bengal test (RBT) (Alton et al., 1988) and modified RBT (MRBT) was done according to (Blasco et al., 1994).

2. Protective activity in mice was done according to (OIE, 2010).

RESULTS AND DISCUSSION

Table 1: Results of toxicity test of the prepared RVF vaccines in mice

Type of vaccine	Toxicity test		
	S/C	I/P	Control
RVF vaccine with *Brucella* HSE	0/10*	0/10*	0/10
RVF vaccine alone	0/10*	0/10*	0/10

* Number of dead mice over survived mice

Table 2: Results of Sterility, safety and potency test of the prepared vaccine.

Type of vaccine	Sterility	Safety	potency
		Lambs*	Adult mice (ED_{50}/ml)
RVF vaccine with *Brucella* HSE	Sterile	0/2	0.003
RVF vaccine alone	Sterile	0/2	0.002

* Safety in lambs: no thermal or clinical symptoms in vaccinated animals.

Table 3: S.N.T. for sheep sera vaccinated by RVF vaccine with or without *Brucella* HSE

Groups of animals	No. of animals	Neutralizing Indices																
		Weeks post vaccination (WPV)																
		0	1	2	3	4	5	6	7	8	10	12	14	16	18	20	22	24
G1	1	0.3	0.9	1.4	1.7	2.1	2.4	2.4	2.5	2.7	2.7	3.0	3.0	3.3	3.3	3.4	3.2	3.0
	2	0.3	0.7	1.7	2	2.4	2.4	2.4	2.4	3.0	3.2	3.4	3.4	3.7	3.7	3.7	3.5	3.4
	3	0.4	1.0	1.4	1.9	2.4	2.7	3.0	3.2	3.0	3.2	3.2	3.4	3.4	3.4	3.2	3.0	3.0
	4	0.3	0.9	1.5	2.2	2.7	2.7	2.7	2.9	2.9	3.0	3.2	3.3	3.4	3.2	3.0	3.0	2.7
	Mean	0.325	0.875	1.5	1.95	2.4	2.55	2.625	2.75	2.9	3.025	3.2	3.275	3.45	3.4	3.325	3.175	3.025
G2	5	0.4	0.6	1.4	1.9	2.4	2.2	2.5	2.5	2.7	2.7	2.7	2.7	3.0	2.7	2.7	2.4	2.4
	6	0.3	0.7	0.7	1.5	2.1	2.4	2.4	2.4	2.4	2.4	2.7	2.7	3.0	2.7	2.7	2.4	2.4
	7	0.4	0.9	1.4	1.7	1.9	2.0	2.4	2.5	2.7	2.7	3.0	3.0	3.2	3.0	3.0	2.7	2.7
	8	0.3	0.7	1.3	1.4	2	2.1	2.5	2.5	2.9	2.9	2.7	3.0	3.4	3.2	3.2	2.7	2.7
	Mean	0.35	0.725	1.2	1.625	2.1	2.175	2.45	2.475	2.675	2.675	2.775	2.85	3.15	2.9	2.9	2.55	2.55
G3	9	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.2	0.4	0.4	0.4	0.3	0.3	0.3
	10	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.2	0.2	0.3	0.3	0.2	0.3	0.3
	Mean	0.35	0.25	0.25	0.3	0.35	0.35	0.3	0.35	0.3	0.3	0.2	0.3	0.35	0.34	0.25	0.3	0.3

G1: sheep vaccinated with inactivated RVF with Brucella HSE
G2: sheep vaccinated with inactivated RVF
G3: control non vaccinated sheep

Nowadays, there is a new trend to use of Gram negative bacteria contain LPS, endotoxins, peptidoglycans and lipoproteins which considered the most potent immnostimulant among cell wall component. In this study, brucella HSE was used as an example for such trend.

Results of toxicity test for the prepared vaccine was carried on adult mice as shown in Table (1) revealed that there was no signs of toxicity or inflammation at the site of the injection.

As shown in table (2), the prepared vaccines were sterile and safe when inoculated in lambs which show no thermal or clinical symptoms and no deaths. In the regard to the potency test in adult mice, ED50 were 0.003 and 0.002/ ml for RVF vaccine with and without Brucella HSE, respectively. The prepared vaccines were within the permissible limit as cited by Randall et al., (1964) who said that ED50 must not be more than 0.02/ ml.

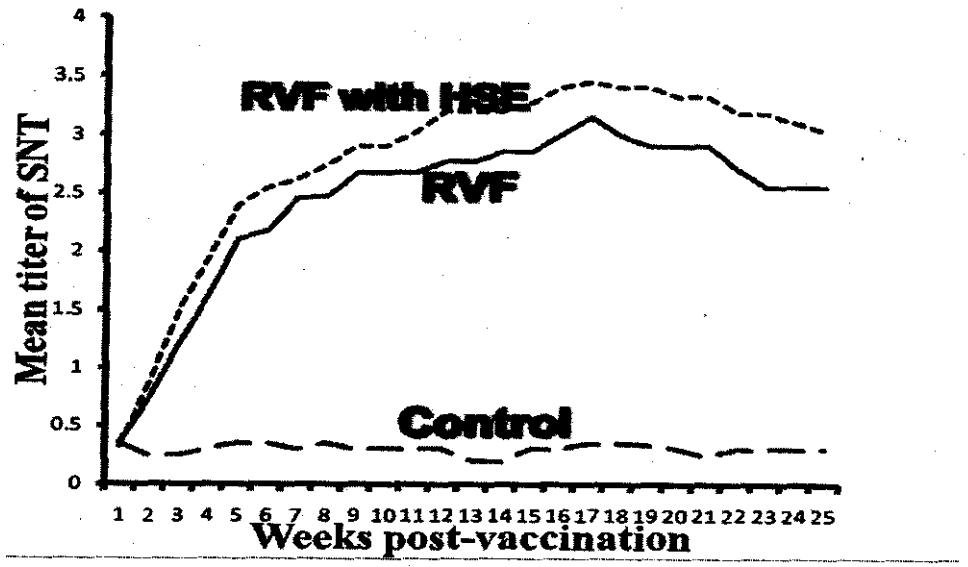

Fig. (1): S.N.T. for sheep sera vaccinated with RVF vaccine with and without *Brucella* HSE

Sheep vaccinated with inactivated RVF vaccine in combination with Brucella HSE gave high titers of antibodies begins from the 3rd week post-vaccination with a titer of 1.95 and reached its peak at 16th week post-vaccination (3.45) while sheep vaccinated with R.V.F. vaccine alone showed protective titre at the 3rd week post-vaccination (1.2) and reached peak of the titer at 14th week post-vaccination (2.85). The protective titer for R.V.F. antibodies was

1.5 that was supported by Pini et al., (1973), Hassan (1998), Zeidan et al., (2004) Naglaa (2005) and Hassan and El-Meneisy (2006) as shown in table (1,2 and 3 and Fig 1).

Table (4) Results of Rose Bengal test for sheep vaccinated with inactivated RVF vaccine with Brucella HSE

Weeks post-inoculation	Serum of Sheep inoculated with Brucella HSE and tested with Rose Bengal test
0	-ve
1	(++++)
2	(++++)
3	(++++)
4	(++++)
5	(++++)
6	(++++)
7	(++++)
8	(++++)
10	(++++)
12	(++++)
14	(++++)
16	(++++)
18	(+++)
20	(++)
22	-/(++++)*
24	-/(++++)*

*Results were negative using Rose Bengal test (RBT)/positive using modified RBT

Table (5): Protective activity of Brucella HSE in adult female Balb/C mice

Groups of mice	Protective activity in female Balb/C mice
Mice inoculated with Brucella HSE	3.2
Mice inoculated with Rev-1 Brucella vaccine	2.6
Control group mice	5.4

Immune responses and protective activity to Brucella HSE were followed up in

this study to detect the efficiency of HSE and ability of this extract to protect against Brucella infection.

Sheep inoculated with 8 mg brucella HSE gave significant antibody titer when tested with RBT for about 20 weeks and when tested with MRBT give positive result for 24 weeks (table 4).

Protection activity conferred by HSE against brucella melitensis biovar 3 (field isolate) was measured in female Balb/C mice using Rev-1 brucella vaccine and unvaccinated groups as control groups. Mean Protection activity conferred by HSE and Rev-1 were 3.3 and 2.6, respectively which is satisfactory according to **(OIE, 2010)** and in comparison with unvaccinated group which was had protective activity 5.4 (table 5).

From thus study we concluded that the use of Brucella HSE with R.V.F. vaccine increase the immune responses of sheep to R.V.F. vaccine and also may give a good immunity to sheep against *Brucella.*

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المستخلص البروتينى للبروسيلا بمحلول الملح الفسيولوجى الساخن كمحفز مناعى مع لقاح حمى الوادى المتصدع فى الاغنام

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*المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية
**معهد بحوث الامصال و اللقاحات البيطرية

تمت هذه الدراسة على ثلاثة مجاميع من الاغنام تم استخدامها لتقييم التحفيز المناعى لمستخرج محلول الملح الفسيولوجى الساخن للبروسيلا وتاثيره على رد الفعل المناعى للاغنام المحصنة بلقاح حمى الوادى المتصدع المثبط. تم تحصين اول مجموعة بلقاح حمى الوادى المتصدع المثبط فقط تحت الجلد اما المجموعة الثانية فقد تم تحصينها بحمى الوادى المتصدع المثبط مع مستخرج محلول الملح الفسيولوجى الساخن للبروسيلا تحت الجلد فى حين لم يتم تحصين المجموعة الثالثة . تم تجميع عينات السيرم التى خضعت لاختبار ال SNTالذى اظهر ان الرد الفعل المناعى للاغنام المحصنة بحمى الوادى المتصدع المثبط مع مستخرج محلول الملح الفسيولوجى للبروسيلا كان اكثر ايجابيا اذا وضع فى الاعتبار نتائج المجموعة المحصنة بحمى الوادى المتصدع المثبط فقط و المجموعة الغير محصنة. تم استخدام مجموعات من الفئران مقابلة لمجموعات الاغنام لقياس السمية ED50 الذى اعطى نتائج مماثلة مع نتائج الSNT . نتائج ال ED50/ مل كانت ٠.٠٠٣ و ٠.٠٠٢ بالنسبة للاغنام المحصنة بحمى الوادى المتصدع المثبط مع مستخرج محلول الملح الفسيولوجى للبروسيلا و للاغنام المحصنة بحمى الوادى المتصدع المثبط فقط على الترتيب. ام بالنسبة لنتائج ال SNT ، اعطت رد فعل ايجابى من الاسبوع الثالث بعد التحصين (١.٩٥) ووصل لاعلى رد فعل مناعى فى الاسبوع السادس عشر بعد التحصين (٣.٤٥) بالنسبة للمجموعة المحصنة بحمى الوادى المتصدع المثبط مع مستخرج محلول الملح الفسيولوجى للبروسيلا اما بالنسبة للمجموعة المحصنة بحمى الوادى المتصدع المثبط فقط فقد اعطت رد فعل ايجابى من الاسبوع الثالث بعد التحصين (١.٢) ووصل لاعلى رد فعل مناعى فى الاسبوع الرابع عشر بعد التحصين (2.85). ايضا تم قياس الرد فعل المناعى للاغنام لمستخرج محلول الملح الفسيولوجى الساخن للبروسيلا باستخدام اختبار الروزبنجال الذى اعطى نتائج ايجابية لمدة٢٤ اسبوع مما يعنى انه يعطى حماية ضد مرض البروسيلا و تم تاكيد هذه النتائج بقياس قوة الحماية فى الفئرن البلبى سى الذى اعطى نتائج مرضية بالمقارنة بالمجموعة المحصنة بلقاح البروسيلا و المجموعة الغير محصنة.