Evaluation of combined vaccines against bovine brucellosis

El-Olemy, G.E.[a], Lobna, M.A. Salem[a], Nashwa, O. Khalifa[a], El-Ayouby, S.M.[b], Manal F. Mohamed[b]

[a] Department of Zoonosis, Faculty of Veterinary Medicine, Benha University, [b] Veterinary Serum and Vaccine Research Institute, Sera and Antigens Department, Abbasia, Cairo

A B S T R A C T

Ten female local breed cows proved to be brucella free (six of them were pregnant, parturated and lactate during the experiment time) were subdivided into two subgroups, the first one vaccinated with combination of HS and OMPs subunit vaccines combined with conjunctival vaccination with Br. abortus strain 19 vaccine and the second group was vaccinated with combination of HS and OMPs subunit vaccines beside vaccination with *Br. abortus* strain RB51 (S/C). From first day post vaccination to 60 days, saliva, vaginal discharge, fecal and milk samples were collected and examined for the presence of the vaccinal strains. Also, blood samples were collected from vaccinated animals and the serum tested serologically using RBPT, MAT and ELISA. In addition, cell mediated immune response was evaluated using Brucellin test. The results revealed that no vaccinal strains was detected in the different body secretions and the humoral immune response of vaccinated cows reached its peak at the 4th week post vaccination then decreased gradually and disappeared at the end of the 10th week. Also, cell mediated immune response revealed that cows vaccinated with the combination of HS and OMPs subunit vaccine combined with conjunctival vaccination with Br. abortus strain 19 vaccine showed remarkable increase in the cell mediated immune response in comparison with in cattle vaccinated with the same combination beside subcutaneous vaccination with *Br. abortus* strain RB51.

Keywords: bovine brucellosis, humoral immune response, Brucellin test

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1. INTRODUCTION

Brucellosis constitutes a major health and economy problem in many parts of the world. The disease causes storm of abortion, retained placenta, orchitis and arthritis in the infected animals (Lamees, 2003, Mariano et al., 2012). In Egypt, it is difficult to differentiate whether the causative agent of bovine brucellosis was due to *Br. abortus* or *Br. melitensis* as they both can cause the disease (Abdel Moghney et al., 2012). Crude *Brucella* membrane protein induced a strong significant level of protection in mice, challenged with *Br. melitensis* virulent strain 16M and the level of protection was similar to that induced by *Br. melitensis* Rev.1 vaccine. A genetic vaccine based on the OMP31 gene can elicit a strong cellular immune response and crude OMPs is a good candidate for use in future studies of vaccination against Bovine Brucellosis (Doosti et al., 2009). Vaccination with hot saline extract (HS) of *Br. ovis* conferred good protection against *Br. ovis* but protection was greatly enhanced by the incorporation of QS- 21 or other adjuvants. Jimenez de Bagues et al. (1994) adjuvanated HS vaccine afforded protection against challenge with *Br. ovis* as good as or better than that provided by attenuated *Brucella melitensis* vaccine strain Rev.1. Therefore, this study was conducted to evaluate *Br. abortus* vaccine and subunit

vaccine of Br. melitensis in vaccination of cows against bovine brucellosis.

2. MATERIAL AND METHODS

2.1. Brucella strains:

Br. melitensis strain H38: A virulent strain was kindly obtained from USDA, Nation Veterinary Laboratories (NSVL), Ames, Iowa, 50010, USA. *Br. abortus* strain 19: A vaccinal strain was kindly obtained from seed strain (obtained from Nation Veterinary Laboratories (NSVL), 1800 Dayton Avenue, Ames, Iowa, 50010, USA. *Br. abortus* strain RB51*:* It was obtained from Professional Biological Company, 4950 Yorj Street, Denver, Colorado 80216.

2.2. Experimental Design and vaccination program:

Ten female local breed cows were obtained from Animal Production Institute, Dokki, Giza. All cows under experiment were proved to be free from internal, external and blood parasites. Also, the sera of these animals were screened for the presence of antibodies against *Brucella* and proved to be free. Six of them were pregnant and parturated then was lactating during the experiment time.

2.3. Samples:

Serum samples: From the first week of vaccination till 10 weeks post vaccination blood samples were collected from all vaccinated animals in sterile MacCartney bottles. Fecal, vaginal and milk samples: They were collected from 1st day post vaccination until 60 days and examined for possible shedding of the vaccinal strain according to Alton et al. (1988).

2.4. Rose Bengal Plate Test and Microagglutination Test:

These were carried out according to Morgan et al. (1969) and Brown et al. (1981) for the evaluation humoral immune response against the vaccines used.

2.5. ELISA test:

It was carried out according to Alton et al. (1988) for the evaluation of the humoral immune response.

2.6. Delayed hypersensitivity test:

It was carried out according to Alton et al. (1988) for evaluation of the cell mediated immunity against the vaccines.

3. RESULTS

Table (1): Results of brucellin test in cows vaccinated with a combination of HS and OMPs subunit vaccine combined with conjunctival vaccination with 4 x 10^9 CFU strain 19 vaccine

Animal No.	Observation time (Hours) post inoculation of brucellin				
	Pre-inoculation	4	24	48	72
1	2.3 mm	No change	2.9 mm	6.8 mm	4.1 mm
2	2.0 mm	No change	2.4 mm	6.4 mm	3.9 mm
3	2.2 mm	No change	2.9 mm	6.7 mm	3.5 mm
Control non-vaccinated	2.0 mm	No change	2.0 mm	2.0 mm	2.0 mm

Table (2): Results of brucellin test in cows vaccinated with a combination of HS and OMPs subunit vaccines combined with 4 x 10^9 CFU RB51 strain vaccine injected subcutaneously

Animal No.	Observation time (Hours) post inoculation of brucellin				
	Pre-inoculation	4	24	48	72
1	2.2 mm	2.4 mm	3.2 mm	5.8 mm	3.6 mm
2	2.0 mm	2.2 mm	2.9 mm	5.8 mm	3.3 mm
3	2.1 mm	2.1 mm	2.9 mm	5.6 mm	3.3 mm
Control non-vaccinated	2.2 mm	2.2 mm	2.2 mm	2.2 mm	2.2 mm

Table (3): Results of RBPT and MAT in cows vaccinated with a combination of HS and OMPs subunit vaccine combined with 4 x 10^9 CFU of strain 19 vaccine inoculated conjunctively

Weeks post vaccination	RBPT %	No. *	Reaction **	MAT
1	100 %	5 (5)	++	83
2	100 %	5 (5)	+++	215
3	100 %	5 (5)	+++	293
4	100 %	5 (5)	+++	302
5	80 %	4 (5)	++	254
6	80 %	4 (5)	++	192
7	80 %	4 (5)	+	107
8	60 %	3 (5)	+	86
9	40 %	2 (5)	+	56
10	20 %	1 (5)	-ve	20

* Number of positive animals. ** Mean degree of the total serum samples test.

Fig. (1): Results of Rose Bengal Plate Test (RBPT) and Microagglutination Test (MAT) of vaccinated cows

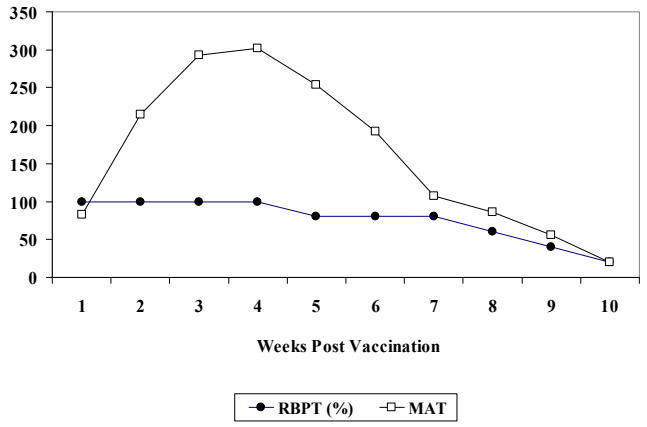


Table (4): Results of ELISA test in cows vaccinated with a combination of HS and OMPs subunit vaccines combined with 4 x 10^9 CFU strain 19 vaccine inoculated conjunctivally

Weeks post vaccination	Optical density of samples 1	2	3	4	5	Mean OD	ELISA Unit
1	0.756	0.783	0.719	0.625	0.879	0.752	25.3
2	0.9645	0.8770	1.0205	1.1725	1.1995	1.047	57.6
3	1.3260	1.0385	1.0515	1.2535	1.1295	1.158	69.7
4	1.6005	1.5675	1.3945	1.6125	1.6080	1.557	113.4
5	1.430	1.441	1.364	1.394	1.344	1.396	95.81
6	1.198	1.151	1.158	1.153	1.239	1.180	72.2
7	0.868	0.862	0.943	0.952	0.991	0.923	44.1
8	0.7465	0.6485	0.6915	0.8775	0.7250	0.738	23.7
9	0.632	0.696	0.641	0.622	0.695	0.657	14.9
10	0.607	0.636	0.600	0.664	0.621	0.626	11.5

N.B. +ve ELISA unit > 20

Fig. (2): Results of ELISA test in cows vaccinated with a combination of HS and OMPs subunit vaccines combined with 4 x 10^9 CFU strain 19 vaccine inoculated conjunctivally

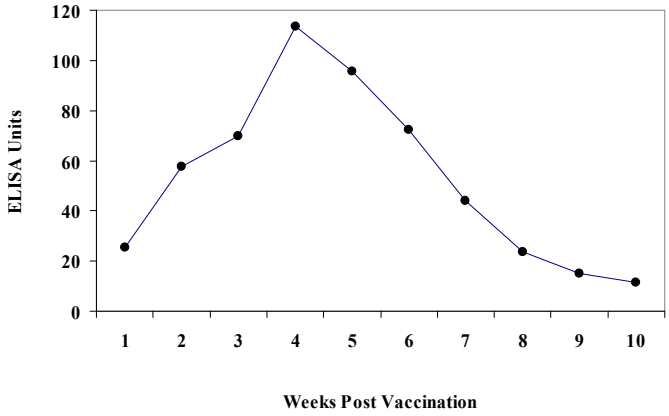


Table (5): Results of Rose Bengal Plate Test (RBPT) and microagglutination of cows vaccinated with combination of HS and crude OMP subunit vaccines combined with subcutaneous vaccination with 4 X 10^{10} CFU of RB51 strain vaccine

Weeks post vaccination	RBPT %	No. *	Reaction **	MAT
1	100 %	5 (5)	+	62
2	100 %	5 (5)	++	117
3	100 %	5 (5)	++	206
4	100 %	5 (5)	+++	287
5	80 %	4 (5)	++	232
6	80 %	4 (5)	+	186
7	80 %	4 (5)	+	97
8	60 %	3 (5)	+	53
9	20 %	1 (5)	-ve	17
10	20 %	1 (5)	-ve	17

Fig. (3): Results of Rose Bengal Plate Test (RBPT) and microagglutination of cows vaccinated with combination of HS and crude OMP subunit vaccines combined with subcutaneous vaccination with 4 X 10^9 CFU of RB51 strain vaccine

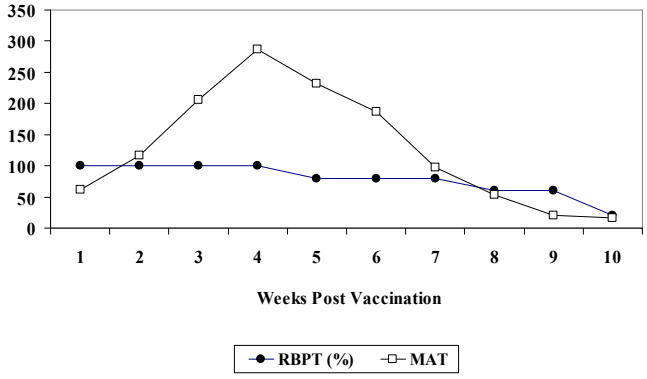
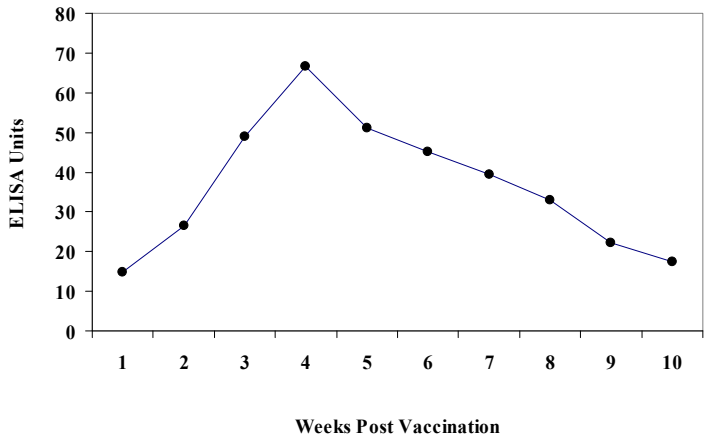


Table (6): Results of ELISA test in cows vaccinated with combination of HS and crude OMP subunit vaccines combined with subcutaneous vaccination with 4 X 10^9 CFU RB51 strain vaccine

Weeks post vaccination	Optical density of samples 1	2	3	4	5	Mean OD	ELISA Unit
1	0.437	0.362	0.344	0.420	0.404	0.393	14.9
2	0.456	0.427	0.440	0.488	0.509	0.464	26.6
3	0.622	0.644	0.612	0.577	0.543	0.600	48.9
4	0.678	0.654	0.704	0.712	0.796	0.709	66.7
5	0.621	0.537	0.621	0.648	0.695	0.613	51.0
6	0.592	0.528	0.521	0.614	0.628	0.577	45.1
7	0.537	0.495	0.488	0.587	0.604	0.542	39.3
8	0.451	0.407	0.517	0.551	0.588	0.503	33.0
9	0.498	0.418	0.377	0.422	0.472	0.437	22.1
10	0.456	0.404	0.462	0.314	0.407	0.409	17.5

Fig. (4): Results of ELISA test in cows vaccinated with combination of HS and crude OMP subunit vaccines combined with subcutaneous vaccination with 4 X 10^9 CFU RB51 strain vaccine



4. DISCUSSION

The present study was aimed to find out the best type of brucella vaccination program which can be used for control of bovine brucellosis in Egypt without or with minimum disadvantage. To achieve this aim, two groups of cows were vaccinated with a combination of oily adjuvanted HS and OMPs subunit vaccine combined with either conjunctival vaccination with Br. abortus strain 19 vaccine in one group or subcutaneous vaccination with Br. abortus strain RB51 in the second group. The shedding of the different vaccinal strains in the body secretions or the milk of vaccinated cows revealed that no vaccinal strains were detected. The obtained results agreed with results obtained previously (Nicoletti, 1984, Lim, 1990, Perez et al. 1995, Olsen et al. 1995, Samartina et al., 2000, Lamees, 2003). The evaluation of the humoral immune response of vaccinated cows was conducted by application of the serological tests on the serum samples as RBPT, MAT and ELISA. The results indicated that all the vaccines used in this study produced antibody responses which began from 1st week post vaccination reaching their peak on the 4th week and decreased gradually and nearly disappeared within 10 weeks (Tables 3, 4, 5, 6) and (Fig. 1, 2, 3). These results are similar to that obtained by Fensterbank et al. (1982), OIE (1996), Olsen et al. (1998), Perez et al. (1995), Alavi Shoushtari and Zeinali

(1995), Nicoletti (1984) and Lim (1990). The results of Brucellin illustrated in Tables (1, 2) showed increased in skin thickness, and reached their maximum level at 48 hours after i/d inoculation of Brucellin. These results agreed with that of Ottosen and Plum (1955), Bercovich and Muskens (1999) and Saegerman et al. (1999).

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