Experimental study of brucella vaccines in mice and guinea pigs, with special references to serological diagnosis of workers in field of vaccine production

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A B S T R A C T

In this study, groups of 150 brucella free white Swiss mice, were inoculated with different oily adjuvanated brucella melitensis subunit vaccines alone or both and then were challenged with Br. melitensis virulent strain for the evaluation of the immune response of each subunit vaccine which was judged by testing this potency through spleen to body weight ratio and the number of brucellae per gram spleen. Also two groups of brucella free guinea pigs were inoculated with a combination of different oily adjuvanated Brucella melitensis subunit vaccines combined with either live attenuated Br. abortus strain 19 vaccine, inoculated conjunctivally or RB51 vaccine injected subcutaneously. Then the animals were challenged with virulent Br. melitensis strain. Humeral and cell mediated immune responses were evaluated. The shedding of the vaccinal strains in the different body secretions was detected along the whole days of the experiment. Eighteen serum samples were collected from occupational group working in the vaccine production and application for serological examination. It was found that the protection level in the different mice animals groups was 70%, while in the vaccinated guinea pigs vaccinated with the combined oily adjuvanated Br. melitensis subunit vaccine combined with strain 19 vaccine, was 90%, while in those combined with RB51 vaccine, the protection level was 85%. In addition, no vaccinal strains were detected in the different body secretions of the vaccinated animals along the whole days of the experiments. Only two cases of the occupational group were positive.

Keywords: bovine brucellosis, humoral immune response, brucella vaccines

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1. INTRODUCTION

Brucellosis a zoonosis of worldwide importance, constitutes a major health and economy problem in many parts of the world (Lamees, 2003). The bacteriological studies revealed the predominance of Br. melitensis biovar 3 in sheep, goat, cattle, buffaloes and camels in Egypt (El-Bauomy, 1983, Montasser et al., 1999 and Manal, 2007). Vaccination is the only method of control and it is suitable for countries with a high incidence of bovine brucellosis (Blasco, 1977 and Eschenbrenner et al., 2002). 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 Rev1 vaccine (Doosti et al., 2009).

Vaccination with hot saline extract (HS) of Br. ovis conferred good protection against Br. ovis and the protection was greatly enhanced by the incorporation of QS-21 (Quillaja Saponaria) or other (Jimenez de Bagues et al., 1994). Conjunctival vaccination with strain 19 vaccine has been used successfully to protect the whole herd exposed to infection without excretion of the microorganism in the different secretions of the animals as it is constricted

in the lacrimal gland (Fensterbank et al., 1987). RB51 are effective to prevent abortion and do not shed in the different body secretions (Olsen et al., 1998 and Barradas-Pina et al., 2012).
Therefore, the aim of study was to make a combination of antigenic portion of Br. melitensis (H38) and Brucella abortus strain 19 and RB51 vaccines and measure the humoral and cell mediated immunity in mice and guinea pigs as a model and detection of them in different body secretions, also detection of brucellosis in high risk groups (veterinarians and technicians).

2. MATERIAL AND METHODS

2.1. Brucella strains:

Br. melitensis strain H38:

A vaccinal strain was kindly obtained from USDA, National Veterinary Laboratories (NSVL) Ames, Iowa, 50010, USA used for preparation of HS (Hot saline extract) and OMP subunit vaccine (outer membrane protein lipopolysaccharide extract).

Br. abortus strain 19:

A vaccinal strain was kindly obtained from seed strain (obtained from National 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 animals:

Guinea pigs and Mice: Groups of Brucella free guinea pigs (180) and mice were obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI).
Animals were vaccinated with combination of HS and OMPs subunit vaccines combined with either conjunctival vaccination with strain 19 vaccine or with subcutaneous vaccination with RB51 vaccine and the serum samples were collected from the 1st week until 10th week post vaccination for serological examination and also the cell mediated immune response was judged using Brucellin test.

2.3. Serum samples:

Animal serum samples:
From the first week of vaccination till 16 weeks post vaccination, blood samples were collected from animals of each vaccinated group in sterile plastic tubes.
Human serum samples: Eighteen serum samples of veterinarians and technicians.

2.4. Faecal, vaginal and nasopharyngeal samples:

Those were collected from 1st day post vaccination until 60 days and examined for the shedding of the vaccinal strains.

2.5. Rose Bengal plate Test and micro-agglutination test:

These were carried out on sera collected from the vaccinated animals according to Morgan et al. (1969) and Brown et al. (1981) for the evaluation of humoral immune response against the vaccines used.

2.6. Delayed hypersensitivity test:

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

2.7. Tube agglutination test:

This was carried out on sera collected from high-risk group (veterinarians and technicians) according to Alton et al. (1988).

3. RESULTS

Table (1): Challenge (potency) test of mice vaccinated with a combination of HS subunit vaccine and crude OMP subunit vaccine and challenged with 2 x 10^5 CFU of *Br. melitensis* H38 virulent strain

Animal No.	Body weight	Spleen weight	S/B ratio	Colonies/Spleen X	logY	Protection
1	23.2	0.37	1.6	647.0	2.4	P
2	24.5	0.36	1.5	450.5	2.2	P
3	25.2	0.25	1.0	698.8	2.4	P
4	22.1	0.33	1.5	170.8	1.8	P
5	22.9	0.27	1.2	186.9	1.9	P
6	25.7	0.31	1.2	305	2.1	P
7	23.6	0.33	1.4	298.5	2.1	P
8	22.8	0.30	1.3	1048.2	2.5	NP
9	23.7	0.21	0.9	835	2.5	NP
10	25.7	0.275	1.1	1050	2.5	NP
Protection %						70 %

P: Protected. NP: Non-Protected.

$$\text{S/B: Spleen weight / Body weight ratio} = \frac{\text{Spleen weight}}{\text{Body weight}} \times 100$$

N.B. According to OIE (2000): X = Number of *Brucella*e per gram spleen. Y = Log (X/logx) = response and protection of mice < 2.5

Table (2): The results of Rose Bengal Plate Test (RBPT) and micro agglutination test (MAT) of G. pigs vaccinated with a combination of HS and OMPs subunit vaccines combined with conjunctival vaccination with strain 19 vaccine followed by challenging with 2 x 10^5 CFU of *Br. melitensis* H38 virulent vaccine

Weeks		RBPT %	RBPT No. *	Reaction	MAT**
	1	0	0 (10)	-ve	20
	2	80	8 (10)	+ve	92
	3	100	10 (10)	++ve	181
Weeks post vaccination	4	100	10 (10)	+++ve	240
	5	100	10 (10)	++++ve	320
	6	80	8 (10)	+++ve	194
	7	80	8 (10)	++ve	166
	8	70	7 (10)	++ve	142
	1	70	7 (10)	++ve	120
	2	60	6 (10)	+ve	52
	3	80	8 (10)	++ve	108
Weeks post challenge	4	100	10 (10)	+++ve	146
	5	100	10 (10)	+++ve	213
	6	100	10 (10)	+++ve	152
	7	100	10 (10)	++ve	104
	8	100	10 (10)	++ve	96
Protection %					90 %

* Number of positive animals. N.B. Number between brackets in the RBPT are the total serum tested. ** Mean degree of positivity of the total serum samples tested. Values of MAT expressed in International unit (IU).

Table (3): The results of Rose Bengal Plate Test (RBPT) and microagglutination test (MAT) of G. pigs vaccinated with a combination of HS and OMPs subunit vaccine combined with a subcutaneous vaccination with RB51 vaccine

Weeks		RBPT %	RBPT No. *	RBPT Reaction	MAT
Weeks post vaccination	1	0	0 (10)	-ve	20
	2	70	7 (10)	+ve	84
	3	80	8 (10)	++ve	97
	4	100	10 (10)	++ve	145
	5	100	10 (10)	+++ve	187
	6	100	10 (10)	+++ve	249
	7	80	8 (10)	++ve	132
	8	60	6 (10)	+ve	84
Weeks post challenge	1	60	6 (10)	+ve	70
	2	50	5 (10)	+ve	66
	3	70	7 (10)	++ve	102
	4	80	8 (10)	++ve	133
	5	100	10 (10)	+++ve	175
	6	100	10 (10)	+++ve	193
	7	90	9 (10)	++ve	127
	8	80	8 (10)	+ve	80
Protection %					85 %

* Number of positive animals. N.B. Numbers between brackets in the RBPT are the total serum tested. ** Mean degree of positivity of the total serum samples tested. Values of MAT expressed in International unit (IU).

Table (4): Serological examination of eighteen human serum samples from people who work in contact with the production and examination of the vaccines

No. of serum sample	RBPT +ve	RBPT %	TAT +ve	TAT %
18	2	11	2	11

4. DISCUSSION

The present study was conducted to find out the best kind of brucella vaccination program which can be used for eradication of bovine brucellosis in Egypt without or with minimum disadvantage. To achieve this aim, groups of (150) mice and (180) guinea pigs were vaccinated with a combination of HS and OMPs subunit vaccines combined with either conjunctival vaccination with strain 19 vaccine or S/C vaccination with RB51 vaccine then the humoral and cell mediated immune responses were evaluated beside detection of the shedding of the vaccinal strains in the different body secretions of the vaccinated animals. The protection level in the vaccinated mice as showed in Table (1) and that in the guinea pigs vaccinated with strain 19 conjunctivally was 90% and that with strain RB51 S/C was 85% as shown in Tables (2, 3). The results agreed with Plommet (1980), Alton et al. (1988), Fensterbank et al. (1982), Jimenez et al. (1994), Harry Glenchur et al. (1963), Cloackaert et al. (2002) and Cassataro et al. (2007). The evaluation of the cell mediated immune responses in vaccinated guinea pigs revealed the occurrence of erythema and slight oedema at the sites of injection reached its highest degree 24 hours after i/d injection of Brucellin as shown in Tables (4, 5). Our results agreed with Alton et al. (1988) and Bercovich et al. (1999). The humoral immune responses were evaluated using RBPT and MAT as shown in Tables

(2, 3) and it indicated that all the vaccinated animals showed antibody responses which began from 1st week post vaccination reaching their maximum level in the 5th and 6th weeks post vaccination and decreased gradually and nearly disappeared at 8 weeks post challenge. The results agreed with Fensterbank et al. (1982), OIE Manual (1996), Alavi-Shoushatri and Zeinali (1995). In addition, studying the shedding of the vaccinal strains in the different body secretion revealed that no vaccinal strains were detected. The obtained results agreed with Nicoletti (1984), Lim (1990), Perez et al. (1995), Olsen et al. (1998) and Lamees (2003). Serum of people at high risk (veterinarians and technicians) was serologically examined by tube agglutination test as shown in Table (6) and it revealed that only two cases of eighteen were positive and it was due to carelessness during handling with the vaccinal strain.

It is concluded that vaccination with HS and OMPs subunit vaccine combined with conjunctival vaccination with *B. abortus* 19 was superior than that combined with subcutaneous vaccination with *B. abortus* strain RB19 in protection against *B. melitensis* infection and abortion. Therefore, it is recommended the important use of combination of HS and OMPs subunit vaccines derived from *B. melitensis* strain H38 combined with conjunctival vaccination with *B. abortus* strain 19 in dose of 4 x 10^9 CFU for controlling of Brucellosis in Egypt.

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