Benha Veterinary Medical Journal 40 (2021) 84-88

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Protective Response of Bivalent Brucella Vaccine with Different Adjuvant in Mice

Ashraf A. Abd El Tawab[1], Lamees A. El- Tantawy[2], Basma M. Hussien[2]

[1]*Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, Egypt*

[2] *Veterinary serum and Vaccines Research Institute*

ARTICLE INFO

Keywords

brucella vaccines
adjuvants
immunogenic test
protection index

Received 25/07/2021
Accepted 31/07/2021
Available On-Line 01/10/2021

ABSTRACT

Brucellosis is one of the most common bacterial zoonotic diseases. There are two live attenuated vaccines used in control programs S19, *B.* rev1 vaccines for cattle, sheep and goats respectively. Some studies had observed one host infected with two different types of brucella. So, in our study, we tried to prepare bivalent vaccine from two vaccinal strains to protect animal from virulent infection. Total of 150 brucella-free mice divided into six groups, the first group was the control one, the second group was vaccinated with strain 19 vaccine, the Third group was vaccinated with the rev1 vaccine, the fourth was injected with the bivalent vaccine without any adjuvant, the fifth was injected with the bivalent vaccine with mantonide Gel 1 (10%), and the sixth was injected with the bivalent vaccine with mantonide oil 206(1:1). The control and vaccinated mice were challenged with virulent strains after 30 days of vaccination, the 2nd group was challenged with *B. abortus* 544, the 3rd group was challenged with *B. melitensis* 16M and the 1st,4th, 5th and 6th were challenged with both strains separately. The immunogenicity of the vaccinated mice was measured after 15 days of challenge with virulent strains. It was found that the protective index of the 4th, 5th and 6th was (2.29, 2.53, 2.66 respectively) in mice when challenged with *B. abortus* 544, and was (2.41, 2.53, 2.68 respectively) in mice when challenged with *B. melitensis* 16M. The prepared vaccines were potent in comparison with control group.

1. INTRODUCTION

In Egypt, brucellosis is still endemic causing large economic losses. It reduces livestock production and reproduction performance by infertility, retention of placenta, metritis, birth of weak calves, stillbirth, abortion especially during the last trimester and 20% reduction in milk production in infected cows suffering from the disease (Asakura et al., 2018 and Lakew et al., 2019). Controlling of brucellosis depends on Test-and slaughter programs in conjunction with vaccination which has great importance. Live attenuated *B. abortus* S19, *B. abortus* RB51 for cattle, and *B. melitensis* Rev-1 vaccine for sheep and goat were licensed to control livestock brucellosis. The aim of vaccination is the reduction of susceptibly individuals in the population. The success of any vaccination program depends mainly on the effectiveness of the vaccine used and its coverage in the target population (Godfroid et al., 2005, Avila-Caldero´n et al., 2013, Elaine et al., 2015 and Lalsiamthara and Lee, 2017). Adjuvant is a chemical substance used to improve the immune response against specific antigens. Addition of Montanide ISA to vaccines achieves the best balance between safety and efficacy. Efficacy of Adjuvants depend on its ability to enhance humoral, or cell mediated immune response. Montanide Gel has a strong ability to fix antigenic proteins probably to its surface. Montanide Gel based vaccine injection will induce the immune response against an antigen. It continuously releases antigen from the injection site, enhancing phagocytosis of the antigen complex with the polymer and inducing pro-inflammatory profile therefore raising the activity of antigen presenting cells (Vialle et al., 2010). some studies recorded the transmission of the Brucella outside Preferred host species in field conditions, which leads to economic losses and signifies a danger to the human. (wareth et al ,. 2014) and some animals infected with more than one type of Brucella at the same time so, the importance of our study came to protect the animals from the most virulent species of Brucella (abortus and melitensis).The aim of this study was to prepare a novel bivalent vaccine to adapt to trans-species infections and protect cattle and sheep from *B. abortus* and *B.melitensis* infections with addition of adjuvants to improve immune response, mice was used as model to measure the immune response of prepared bivalent vaccine and comparing the Immunogenicity of prepared vaccine and ordinary vaccines in mice.

2. MATERIAL AND METHODS

2.1. Materials:

2.1.1. Bacterial strains:

B. abortus S19 strain is a smooth attenuated *B. abortus* (strain 19) ,*B. melitensis* Rev 1strain: is a smooth attenuated

* Corresponding author: ashraf.awad@fvtm.bu.edu.eg

classical vaccine and Challenge strains: *B. abortus 544* and *B.melitensis* 16M were obtained in lyophilized ampoules from Nation veterinary laboratories (NSVL), Ames, Iowa, USA.

2.1.2. Adjuvants:

Mantonide Oil 206 (add to vaccine 1:1 ratio) and Mantonide Gel 01(add to vaccine 10%) SEPPIC, France

2.1.3. laboratory Animals

Mice: one hundred and fifty (n= 150) healthy mice were between 5 and 6 weeks of age, obtained from veterinary serum and vaccine research Institute farm, Abbasia, Cairo (VSVRI). Mice were feed on a balanced diet and water; all efforts were made to minimize animal suffering. All animals proved to be brucella free by serology before use. Mice were divided into six groups as shown in fig (1)

2.2. Experimental design:

one hundred and fifty (n= 150) healthy mice were divided into six groups as showed in figure (1). Group1 contained thirty mice (n=30), each mouse was injected with 0.1 ml of phosphate buffer saline. Group 2 contained fifteen mice (n= 15), each mice was injected with 0.1 ml of B.*abortus* s19 vaccine($1x10^5$). Group 3 contained fifteen mice (n= 15), each mice was injected with 0.1 ml of *B.melitensis* rev1 vaccine($1x10^5$). Group 4 contained thirty mice (n=30), each mouse was injected with 0.1 ml of bivalent vaccine($1x10^5$). Group 5 was contained thirty mice (n=30), each mouse was injected with 0.1 ml of bivalent vaccine($1x10^5$) with mantonide gel (10%). Group 6 was contained thirty mice (n=30), each mouse was injected with 0.1 ml of bivalent vaccine($1x10^5$) with mantonide oil 206 (1:1).

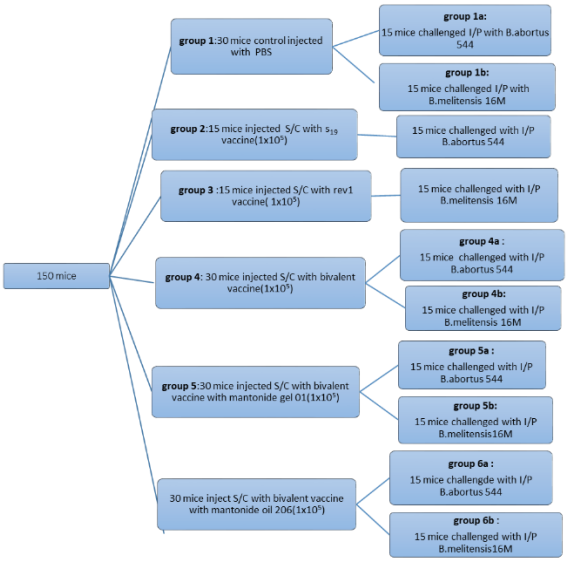

Figure 1 The experimental design and grouping of mice in our study

2.3. Vaccine preparation: OIE (2012)

Brucella abortus S19 and *B. melitensis* Rev.1 seeds were cultured in roux bottles and was incubated at 37 for 3-5 days. Brucella cells were harvested from different roux bottles by adding 10 ml of normal saline in each roux and mix gentily. brucella cells were collected in clean septic containers then viable brucella cells were counted to each strain and adjusted to ($1x10^9$). we mixed equal amount of two prepared vaccines for injecting in mice of Group 4**.** we mixed equal amount to two prepared vaccines and were added mantonide gel 01 (SEPPIC) 10% for injecting in mice of Group 5. we were mixed equal amount to two prepared vaccines and were added mantonide oil 206 (SEPPIC) 1:1 for injecting in mice of Group 6**.**

2.4. Evaluation of prepared Brucella vaccine: It was performed according to Office International des Epizooties (2008) including sterility, safety, viable count of brucella and potency tests.

2.4.1. Sterility test: the sterility tests were done to assure that the prepared vaccines are free from any biological contaminant. 0.2 ml of prepared vaccine was inoculated into thioglycollate broth, tryptose agar, sabauroud ś dextrose and macconkey agar then incubated at 37^0c for 14 days and the plates were examined daily for any growth.

2.4.2. Safety test: Groups of at least ten guinea-pigs are given intramuscular(I/M) injections of doses of vaccine diluted in PBS, pH 7.2, to contain 5×10^9 viable organisms. The animals should show no obvious adverse effects and there must be no mortality.

2.4.3. Viable count of the vaccine:

-Suspension of brucella vaccines were prepared then 1ml of prepared vaccine was withdrawn using sterile pipette and added to 99ml of the peptone water bottles, then ten folded dilutions were done until 10^{-7}. 0.1 ml of adequate dilutions of the vaccine were inoculated in at least 5 plates of trypticase soya agar then spread with a sterile glass, wire or plastic spreader. The plates were incubated at 37^o c for 4 days. the number of colony were counted by colony counter and number of viable organisms were calculated per 1ml vaccine culture. Number /ml = average of number in plates x10 x dilution.

2.4.4. Potency test:

Propagation of the viable virulent brucella challenge strain: (Alton et al. (1988).

Slopes of Trypticase soya agar (DIFCO) were inoculated with *B.abortus* 544and *B.melitensis* 16M,incubated at 37^0c for 48 hours then to each slopes ,3ml of sterile saline was added, left for 10 minutes, then rolled until all brucellae were suspended.

Viable count of brucella challenge strain:

The brucella challenge strain suspensions was counted and adjusted to contain $2x10^5$ CFU/ml

Challenge test: Test was performed at 30-day post vaccination. Mice were challenged with virulent strains ($2x10^5$). Group2 (n=15) of mice was challenged with the adjusted virulent $2x10^5$ CFU/ml *B.abortus* 544 (I/p) and group 3($n=10^5$) of mice was challenged with the adjusted virulent $2x10^5$ CFU/ml *B.melitensis* 16M (I/p).Group 1,4,5 and 6 divided into two group each group was contained (n=15) of mice. Groups 1a,4a,5a and 6a were challenged with the adjusted virulent $2x10^5$ CFU/ml *B.abortus* 544 (I/p) ,but groups 1b,4b,5b and 6b was challenged with the adjusted virulent $2x10^5$ CFU/ml *B.melitensis* 16M (I/p) as showed in figure (1).

Brucella spleen count:

Test was performed 15 days after virulent strains injected challenge ten (n=10) of vaccinated mice from each group were weighed and slaughtered. The spleens were removed aseptically and weighed, and any lesions were noticed. Each spleen was grinded in sterile tissue grinder with 9 volumes of sterile saline solution. Three serial tenfold dilutions (1/10, 1/100 and 1/1000) of each homogenate made in the same diluent. 0.2 ml of each dilution was spreaded in Trypticase soya agar (DIFCO) plates and incubated for 4-7 days.

The brucella colonies and the number per gram spleen were counted. (X= number of Brucella for spleen) then calculated Y =Protective average according to the following formula:

Y= log (x / log x). OIE considers a vaccine to be protective when it has a protective activity less than 2.5.

3. RESULTS

3.1. Evaluation of prepared Brucella vaccine:

The results of quality control of the prepared vaccine indicated that it was free from any contaminants as regards to safety test where the prepared vaccine did not show any abnormalities or adverse reactions during the observation period among the inoculated mice.

3.2. Results of potency test, protection level and protection index in mice vaccinated with different bivalent vaccines comparison with B. abortus (S19):

Results of potency test, protection level and protection index in mice vaccinated with different bivalent vaccines comparison with *B. abortus* (s19). Protective activity was measured by average of Brucella spleen count among the vaccinated mice. The current results indicated that the prepared vaccine showed acceptable degree of potency, it presented reduced pathogen colonization for virulent Brucella (16M or 544). Throughout vaccinated mice. Protection level in mice vaccinated with s19 vaccine, bivalent without adjuvant, bivalent vaccine with mantonide oil ISA 206(1:1) and bivalent vaccine with mantonide Gel 01(10%) with S/C route against challenge with *B.abortus* 544 is reached (80%,80%,90%,90% , respectively). protective index in mice vaccinated with s19 vaccine, bivalent without adjuvant, bivalent vaccine with mantonide oil ISA 206(1:1) and bivalent vaccine with mantonide Gel 01(10%), when challenged with *B. abortus* 544 challenge was in range (2.19, 2.29, 2.66,2.53) respectively as it was showed in Table (1) and Figure (2). means that bivalent vaccine without adjuvant gave the same protection level of monovalent vaccine S19 vaccine but adding of mantonide adjuvant led to increased potency and immunological response against virulent strains (*B. abortus* 544) and increase protection index.

Table 1 Results of potency test and protection level in mice vaccinated with different bivalent vaccines comparison with B. abortus (S19).

Vaccine N=10	Challenge strain	X(mean)*	Y (mean)**	Protection level (%)***	Protective Index****
Non vaccinated (control group)	B.abortus 544	UC	4.5	0	
S19	B.abortus 544	556.3	2.31	80	2.19
Mix vaccine	B.abortus 544	434.5	2.21	80	2.29
Mix vaccine With Mantonide gel 01 (10%)	B.abortus 544	224.3	1.97	90	2.53
Mix vaccine With Mantonide oil 206 (1:1)	B.abortus 544	152.6	1.84	90	2.66

*X=number of brucella per gram spleen
**Y=log (x/log X) =protection of mice <2.5
***Protection %= (number of protected mice – total number of mice) X 100
****Protective Index= (Mean Y negative control)- (Mean Y vaccinated)

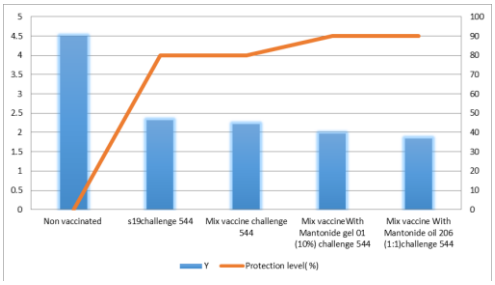

Figure 2 Results of potency test and protection level in mice vaccinated with different bivalent vaccines comparison with B. abortus (S19).

3.3. Results of potency test, protection level and protection index in mice vaccinated with different bivalent vaccines comparison with B. melitensis (rev1):

Protection level in mice vaccinated with REV1 vaccine, bivalent vaccine without adjuvant, bivalent vaccine with mantonide oil ISA 206(1:1) and bivalent vaccine with mantonide Gel 01(10%) with S/C route against challenge with *B. melitensis* 16M is reached (70%,70%,80%,70%, respectively). protective index in mice vaccinated with rev1 vaccine, bivalent vaccine without adjuvant, bivalent vaccine with mantonide oil ISA 206(1:1) and bivalent vaccine with mantonide Gel 01(10%) when challenged with *B. melitensis* 16M challenged was in range (2.35, 2.41, 2.68,2.53), respectively as it was showed in Table (2) and Figure (3). That means the bivalent vaccine without adjuvant gave the same protection level of monovalent vaccine rev1 vaccine but adding of mantonide adjuvant lead to increase protection index. against virulent strains (*B. melitensis*16M).

Table 2 Results of potency test and protection level in mice vaccinated with different bivalent vaccines comparison with *B. melitensis* (Rev1).

Vaccine N=10	Challenge strain	X (mean)*	Y (mean)**	Protection level (%)***	Protective Index****
Non vaccinated (control group)	B.abortus 544	UC	4.7	0	
Rev1	B.abortus 544	626.7	2.35	70	2.35
Mix vaccine	B.abortus 544	536.4	2.29	70	2.41
Mix vaccine With Mantonide gel 01 (10%)	B.abortus 544	440	2.17	70	2.53
Mix vaccine With Mantonide oil 206 (1:1)	B.abortus 544	340	2.06	80	2.68

*X=number of brucella per gram spleen
**Y=log (x/log X) =protection of mice <2.5
***Protection %= (number of protected mice – total number of mice) X 100
****Protective Index= (Mean Y negative control)- (Mean Y vaccinated)

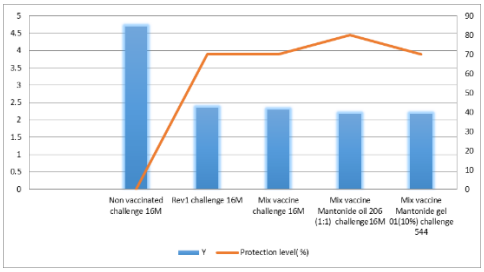

Figure 3 Results of potency test and protection level in mice vaccinated with different bivalent vaccines comparison with *B. melitensis* (Rev1).

4. DISCUSSION

In Egypt, immunization with brucella vaccine is the main control strategies, accompanied by the slaughter of infected animals with positive serological results (Refai, 2002). S19 and Rev1 vaccines are still the most effective vaccines against bovine and caprine brucellosis. (Schurig et al.,2002, Elain et al.,2015). Mixed breeding of cows, buffaloes, sheep and goats has increased the risk of brucellosis where small ruminants act as primary hosts for *B. melitensis* and cattle is spillover host. (El-Wahab et al. 2019). in this study, 150 mice were subdivided into 6 groups as were showed in Figure (1). The protection level and potency test of different vaccinated groups was judged by result of mean Y =log (number of brucella per gram spleen/log number of brucella per gram).

The Protection level and protection index in mice vaccinated with s19 vaccine, bivalent without adjuvant, bivalent vaccine with mantonide Gel 01(10%) and bivalent vaccine with mantonide oil ISA 206(1:1) when challenged with *B.abortus* 544 were showed that the bivalent vaccine without adjuvant gave the same protection level of monovalent vaccine S19 vaccine, but adding of mantonide adjuvant increased potency and immunological response against virulent strains (*B. abortus* 544) and protection index as showed in (Table 1, Figure 2)

On another hand , the Protection level and protection index in mice vaccinated with Rev1vaccine, bivalent without adjuvant, bivalent vaccine with mantonide Gel 01(10%) and bivalent vaccine with mantonide oil ISA 206(1:1) when challenged with *B. melitensis*16M were showed that the bivalent vaccine without adjuvant gave the same protection level of monovalent vaccine rev1 vaccine, but adding of mantonide adjuvant increased immunological response against virulent strains (*B. melitensis* 16M) and increase protection index. as showed in (Table 2, Figure 3)

That means the adjuvated antigen gave a stronger and better response than antigen alone , Oil based vaccines generate a high immune response so, our study results agreed with more studies as Deville et al., (2011) who obtained that the Oil based vaccines generate a rapid, high and long-lasting immune response, Dara et al., (2013) who obtained that mantonide oil adjuvant had the capability for generating a rapid, high and long-lasting immune response when added on FMD (foot and mouth disease) vaccines, Abido et al, (2020) Who obtained that the addition of mantonide oil ISA 206 in preparation of rabbit hemorrhagic disease virus(RHDV) vaccine improves immunogenic effect and increase humoral immunity and Ehab et al, (2015) Who obtained that addition of mantonide ISA oil 206 as adjuvant in preparation of FMD vaccine improved immunogenic effect.

Our study found that addition of mantonide gel 01 induced high immunogenic effect due to highly antigenic response of mantonide by Enhancing phagocytosis which increase antibodies response which agreed with Abdel El-Rahman et al, (2020) Who found that addition of mantonide gel 01 as adjuvant in preparation of rift valley vaccine induces high immunogenic effect and prolonged immunity.

Our study found that bivalent vaccine without adjuvant give the same protection percentage of monovalent vaccine that agreed with Kamaraj et al, (2008) who obtained that *B. abortus* S19 alone and in combination with infectious bovine rhinotracheitis(IBR) vaccine gave the same protection in mice post challenge but disagreed with El-jakee et al, (2020) Who obtained that Vaccination with bivalent (RHDV) vaccine show higher antibody titer and protection 90% than monovalent vaccine which protection 80% against Pasteurella multocida challenge.

5. CONCLUSION

We concluded that bivalent vaccine (s19 and rev1) gave the same of the protection monovalent S19 vaccine (80%,80% respectively) But the adding of adjuvants (mantonide oil 206 or mantonide GEL 01) were increased the protection to 90%. On the other hand, the bivalent vaccine, bivalent vaccine with mantonide Gel 01 gave the same of the protection of the monovalent rev1 vaccine (70%,70%,70% respectively), the adding of mantonide oil 206 increased the protection to 80%. Finally, the results of our study showed that bivalent brucella vaccine can protect mice from virulent strains even *B. abortus or B. melitensis*, adding of mantonide gel or oil 206 improve protection index against virulent strains and the bivalent vaccine with mantonide oil 206 is the best prepared vaccine and can use in brucellosis control programs in cattle and sheep.

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