Avicenna Journal of Clinical Microbiology and Infection
doi:10.34172/ajcmi.2019.17 2019 August;6(3):95-99 http://ajcmi.umsha.ac.ir

Original Article

Evaluation of Humoral Immune Responses of Sheep Vaccinated With Razi Institute Rev.1 Brucellosis Vaccine in Comparison to a Spanish (CZV) Rev.1 Vaccine

Sajad Doostdari[1], Pezhman Mahmoodi[1*], Abdolmajid Mohammadzadeh[1], Abolfazl Khafri[2]

[1]Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[2]Department of Bacterial Vaccines Quality Control, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

*Corresponding author:
Pezhman Mahmoodi;
mahmoodi_pezhman@basu.ac.ir;
Tel: +988134227350; Fax: +988134227475

Received: 10 Mar 2019
Accepted: 13 July 2019
ePublished: 31 Aug 2019

Abstract

Background: Prevention of brucellosis in humans is based on the vaccination of animals. Given that Rev.1 vaccine is one of the most effective vaccines for preventing and controling brucellosis in sheep and goats, the present study was conducted to evaluate and compare humoral immune responses of sheep against Razi Institute and Spanish (CZV) Rev.1 brucellosis vaccines.

Methods: To do the study, 6 sheep were prepared and divided into 2 groups, and blood samples were then collected on day zero. The animals of each group were subcutaneously vaccinated with one dose of Razi Institute and Spanish (CZV) Rev.1 vaccines followed by collecting blood samples on days 30, 60, 90, 120, 150, and 180 post vaccination. Sheep serum samples were then tested using Rose Bengal, Wright, and 2-Mercaptoethanol assays and the data were statistically analyzed.

Results: The results showed that the highest titers of Wright and 2-Mercaptoethanol tests were observed 30 days after vaccination. However, no statistically significant difference was observed between humoral immune responses of sheep vaccinated with either Razi Institute or Spanish (CZV) Rev.1 vaccines ($P>0.05$).

Conclusions: Given the similar results of both vaccines in stimulating the humoral immune system in the target species and the indigenous Razi vaccine production technology, as well as its lower price compared to the imported one, this native vaccine can certainly be used for immunizing livestock in our country. This can ensure the country's independence, boost national vaccine production, and prevent the outflow of currency.

Keywords: Sheep brucellosis vaccine, Rev.1, Razi, Humoral responses

Background

Brucellosis is one of the common diseases between humans and animals, and is associated with fever in humans and abortion in animals (1,2). The bacterial causative agent is a facultative intracellular gram-negative coccobacillus called *Brucella* (3). The *Brucella* genus contains 6 classical species including *B. melitensis, B. abortus, B. ovis, B. canis, B. swiss,* and *B. neotome* (4). Brucellosis is a major public health concern which causes enormous economic losses, including high costs for treatment and inactivity in the community. There is no effective vaccine to prevent this disease in humans and therefore, efforts have focused on preventive measures and infection control in livestock, since this can reduce its incidence in humans. The most effective way to avoid brucellosis in animals is vaccination. In Iran, several vaccines have been used to vaccinate livestock. For example, IRIBA live attenuated vaccine is used to immunize cattle. However, as suggested by the World Organization for Animal Health (OIE), vaccination of sheep with Rev.1 live attenuated vaccine is the most important strategy for the prevention of disease incidence in sheep (5). In addition, the positive effect of this vaccine on the Iranian sheep and goat breeds in Iran climatic conditions has been demonstrated by the researchers of the country in collaboration with the OIE, as the prevalence of the disease has been reduced from 45% to 1.8% with the production of Rev.1 vaccine and the beginning of vaccination programs since 1962 (6). Consequently, the present study was conducted to investigate and compare humoral immune responses of sheep against Razi Institute Rev.1 vaccine, as a completely native vaccine produced in Iran, and a Spanish Rev.1 vaccine (CZV), as a well-known standard imported vaccine.

Methods

Preparation of Serum Samples and Vaccination Initially, 6 sheep of 4- to 6-month-old, which were not previously inoculated with brucellosis vaccine, were randomly selected from Kurdan Station of Razi Vaccine and Serum Research Institute, Karaj, Iran. After 3 days of quarantine, the sheep were randomly divided into two groups and then blood samples were collected on day zero. Afterwards, blood samples were incubated at 37°C for one hour and serum samples were collected by centrifugation at 2000 rpm for 10 minutes and frozen at -20°C until laboratory examinations.

On the other hand, the bacterial cell count of Rev.1 vaccines (Razi and Spanish vaccines) was calculated by colony count method. Thereafter, one dose of Razi and Spanish (CZV) Rev.1 brucellosis vaccines containing 2×10^9 CFU/dose attenuated *B. melitensis* was subcutaneously inoculated to each of the 3 sheep in either group. Blood samples were collected again on days 30, 60, 90, 120, 150, and 180 after vaccination and sera were collected and stored at -20 °C.

Examinations of Serum Samples

Rose Bengal, Wright, and 2-Mercaptoethanol (2-ME) tests were used to evaluate humoral responses of the vaccinated sheep.

Rose Bengal test: To perform this test, a drop of serum (~50 µL) was mixed with a drop (~50 µL) of Rose Bengal antigen on a glass slide and the occurrence of agglutination was monitored for up to 4 minutes under light (7).

Wright test: Serial dilutions from 1/10 to 1/640 were prepared by mixing the serum samples and phosphate-buffered saline (PBS) in tubes. Then, 0.5 mL of Wright antigen (Razi Institute) was added to all tubes, and after covering the tubes with Parafilm, the tubes were incubated at 37°C for 21 hours. Finally, the results were read under light (7).

2-ME test: First, 0.2 mL of sheep serum samples was mixed with 0.3 mL of PBS and 0.5 mL of 2-ME and the mixture was vortexed and incubated at 37°C for 1 hour. After incubation, the same procedure was done for the samples and serial dilutions from 1/10 to 1/640 were prepared by mixing 2-ME treated sera and PBS in tubes. Then, 0.5 mL of Wright antigen (Razi Institute) was added to all tubes, and after covering the tubes with Parafilm, they were incubated at 37°C for 21 hours. Finally, the results were read under light (7).

Statistical Analysis

The data were analyzed using Fisher's exact test and *t*-test in SPSS software (version 16). $P\leq0.05$ was considered to be statistically significant.

Results

The results of Rose Bengal test were positive for all of the serum samples taken up to 60 days after vaccination. However, some serum samples were identified as negative in this experiment from day 120 after vaccination. Full details of the results are presented in Table 1.

On the other hand, the results of Wright test showed that Wright titers of the serum samples of sheep vaccinated with Rev.1 vaccines were decreased 60 days after vaccination. The titers of serum samples for each vaccine are presented in Table 2.

According to the results presented in Table 2, the highest titers of serum samples for both vaccines were those obtained up to 60 days after vaccination. Intergroup *t* test results indicated that there was no significant difference between the serum titers calculated in the Wright test for sheep vaccinated with either Razi or Spanish (CZV) Rev.1 vaccines ($P>0.05$).

Serum samples which were identified as positive in Wright test were analyzed by 2-ME test. Since the disulfide bonds in IgM structure are destroyed by 2-ME in this experiment, the IgG antibody titer is obtained. The results of this experiment showed that the decrease in

Table 1. The Results of Rose Bengal Test for Serum Samples

Days after Vaccination	No. of Positive Samples Rev.1-R Vaccine (%)[a]	No. of Positive Samples Rev.1-C Vaccine (%)[b]	Total (%)
0	0 (0)	0 (0)	0 (0)
30	3 (100)	3 (100)	6 (100)
60	3 (100)	3 (100)	6 (100)
90	3 (100)	2 (66.7)	5 (83.3)
120	2 (66.7)	0 (0)	2 (33.3)
150	0 (0)	0 (0)	0 (0)
180	0 (0)	0 (0)	0 (0)

Note. [a] Razi Institute Rev.1 vaccine; [b] Spanish (CZV) Rev.1 vaccine.

Table 2. Titers of Serum Samples in Wright Test for Each Vaccinated Sheep

Days After Vaccination	Sheep Vaccinated With Rev.1-R vaccine [a]			Sheep Vaccinated With Rev.1-C vaccine [b]		
0	-	-	-	-	-	-
30	160	160	160	160	160	160
60	160	160	160	160	160	160
90	20	20	40	40	40	40
120	20	20	20	20	20	20
150	20	20	20	20	20	20
180	20	20	20	20	10	10

Note. [a] Razi Institute Rev.1 vaccine; [b] Spanish (CZV) Rev.1 vaccine.

the titers of most of the serum samples started on day 30 after vaccination (Table 3).

The results of 2-ME test revealed that the highest titers for both vaccines belonged to sera obtained 30 days after vaccination. Similar to the results of Wright test and as shown in Figure 1, the serum titers followed a decreasing trend after day 30 of vaccination. However, the intergroup *t* test showed that there was no significant difference between the data obtained from 2-ME test for Razi Institute and Spanish (CZV) Rev.1 vaccines ($P > 0.05$).

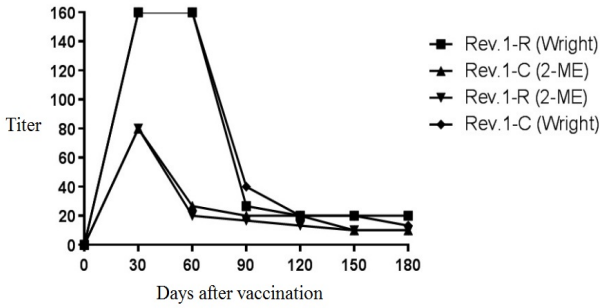

Figure 1. Comparison of the Downward Trend of Mean Serum Titers of Sheep Following Vaccination With Razi Institute (Rev.1-R) and Spanish (Rev.1-C) Rev.1 Vaccines in Wright and 2-ME Tests.

Discussion

Although Rev.1 is the most commonly used vaccine for the prevention of brucellosis in sheep and goats, little information is available on how and to what extent the immune system responds to the vaccine. In addition, existence of different variants of the Rev.1 vaccine doubles the necessity of evaluating the immune responses following the administration of these vaccines.

Razi Institute Rev.1 vaccine is a brucellosis vaccine which is produced locally at a lower price compared to its imported counterpart (Spanish CZV Rev.1), and if there is no difference in the effect of these 2 vaccines, Razi Rev.1 vaccine can definitely and completely meet the country needs and prevent currency outflows for vaccine imports. As a result, the present study aimed to evaluate and compare humoral responses of sheep following the inoculation of Razi Institute and Spanish (CZV) brucellosis Rev.1 vaccines using Rose Bengal, Wright, and 2-ME tests.

Rose Bengal test is a simple, fast, and reliable assay which is performed as a screening test at the herd level, and many of the researchers have shown that this test can detect *Brucella* infection in a preliminary examination (2). The test is a seroagglutination test which retains its preference because of specificity. An acidic solution contains stained *B. abortus* which is used as an antigen source in this assay (8). The results of this test showed that following inoculation of Spanish Rev.1 vaccine (CZV), all of the serum samples were positive 30 and 60 days after vaccination. However, 90 days after vaccination, 83.3% of the samples were positive and from 150 days, the results of Rose Bengal test were negative for all sera. In case of Razi Rev.1 vaccine, the results of Rose Bengal test were positive for all serum samples up to 90 days after vaccination. However, for those sera collected 120 days after vaccination, 66.7% of the samples were positive, and from the 150 days after vaccination, no sample was found to be positive. Nevertheless, as indicated by the statistical analysis (Fisher's exact test), no significant difference was observed in this regard. Similar studies have been carried out elsewhere in the world. For example, Blasco et al reported that following inoculation of Rev.1 vaccine, the results of Rose Bengal test were positive for 98% of the cases in the first week and 80% of them in the eighth week after the inoculation. While, only 28% of the samples had a positive reaction 22 weeks (154 days) after inoculation (9). In the study of Díaz-Aparicio et al, 80% of vaccinated sheep showed positive reaction in Rose Bengal test 6 months after inoculation with Rev.1 vaccine (10). Stournara et al reported that Rose Bengal test of all specimens was positive 21 days after the inoculation of 3- to 6-month-old sheep as well as adult sheep with Rev.1 vaccine, which is consistent with the results of the present study. In addition, they recorded that the results of the Rose Bengal test were negative for 3- to 6-month-old sheep 4 months after vaccination, which is similar to the results obtained from the inoculation of sheep with Spanish Rev.1 vaccine (11). Shome et al showed that 6 months after the inoculation of sheep with Rev.1 vaccine, 95% of samples had positive reaction as indicated by Rose Bengal assay (12). The study of Benkirane et al revealed that following inoculation of Rev.1 vaccine, the results of Rose Bengal test for all samples were positive up to 2 months after vaccination, which is in agreement with the results of the present study (13). Furthermore, the results of all samples in the above study were negative 140 days after vaccination, which is consistent with the results of Rose Bengal tests for both Razi and CZV Rev.1 vaccines.

Table 3. Serum Titers Obtained in the 2-ME Test

Days After Vaccination	Sheep Vaccinated With Rev.1-R vaccine [a]			Sheep Vaccinated With Rev.1-C vaccine [b]		
0	-	-	-	-	-	-
30	80	80	80	80	80	80
60	20	20	20	40	20	20
90	20	10	20	20	20	20
120	20	10	10	20	20	20
150	10	10	10	10	10	10
180	10	10	10	10	10	10

Note. [a] Razi Institute Rev.1 vaccine; [b] Spanish (CZV) Rev.1 vaccine.

Wright seroagglutination test is also one of the most common serological tests for the diagnosis of brucellosis. This method has been a basic test for the detection of *Brucella* infection in early brucellosis eradication programs in countries such as Norway, Denmark, and Sweden (7). However, serious doubts have been raised about the quality of this seroagglutination test because it is immunologically possible to have nonspecific agglutinins against *Brucella,* and also most IgG antibodies are not capable of agglutinating antigens. Consequently, false negative results may be observed (2,14). In the present study, after inoculation of sheep with Rev.1 brucellosis vaccines, the titers of serum samples in Wright test were increased from zero in day 0 to 160 after 1 month in both groups. In a study carried out by Gharib Mambini et al, following inoculation of the Razi Institute Rev.1 vaccine, Wright titers were reported 120 after 1 month, which is lower than the titers recorded in our experiment (15). The discrepancy in the reported titers may be attributed to the number of bacterial cells in the inoculated vaccines, as in that study, a dose of Razi Rev.1 vaccine containing 1-3×10^9 CFU/dose was inoculated which gave a relatively wide range, whereas, 2×10^9 CFU/dose of vaccine was accurately calculated and inoculated to sheep in the present study. Aldomy et al reported that Wright titers were 80 for sheep, 24 weeks post vaccination with Rev.1, while in the present study, the Wright titers for both vaccines were 160 and 20 after the first and fifth months, respectively (16). Benkirane et al documented that after 7 months of inoculation with Rev.1 vaccine, Wright test results were negative (13). The results of this study also showed that antibodies were still detectable 6 months after vaccination. In the study performed by Shome et al, 6 months after the inoculation of Rev.1 vaccine, 65% of sheep had Wright titers higher than 40 which were different from our results. Because after the same time, for Spanish Rev.1 (CZV), 66.7% had a titer of 10 and 33.3% had a titer of 20, and about Razi Rev.1, all samples had a titer of 20 (12).

2-ME experiment was done to investigate the reaction of IgG molecules in serum samples since 2-ME breaks down the disulfide bonds in the IgM structure, thereby destroying the IgM in the serum and the dominant antibody remaining in the serum will be IgG. The results indicated that the titers of serum samples in 2-ME assay were lower than those in Wright test and 2-ME titers were 80 for both sera groups 1 month after vaccination, while Wright titers obtained at this time were 160 for serum samples. The results of 2-ME test showed that IgG antibodies against *B. melitensis* were present in these samples up to 6 months post inoculation. However, the highest amount of agglutinin IgG was found in the serum samples taken 1 month after vaccination, though the concentration of agglutinin IgG decreased over the time. According to the study of Gharib Mambini et al, following inoculation of Razi Rev.1 vaccine, 2-ME titers of sera were reported 40 after 1 month, which was lower than the titers recorded in our study (15).

Conclusions

In general, many factors such as mass of inoculated vaccine, breed and age of studied sheep, conditions of livestock keeping, individual differences, and the like may cause discrepancies in the reported titers. However, the immune response appears to follow a relatively similar pattern in all studies. The results of the serological assays including Rose Bengal, Wright, and 2-ME tests in both animal groups, each containing 3 sheep, revealed that there was no significant difference in sheep humoral immune responses after subcutaneous inoculation of the animals with one dose of Razi and Spanish Rev.1 (CZV) vaccines containing 2×10^9 CFU/dose attenuated *B. melitensis*. Based on these experiments, it can be concluded that the Spanish Rev.1 vaccine (CZV) is not superior to the Razi Rev.1 vaccine in the stimulation of humoral immune responses of sheep. Therefore, it is recommended that vaccines such as Spanish Rev.1 (CZV) be imported into the country as minimally as possible and the Razi Rev.1 Vaccine be used more frequently since this vaccine is produced in our country, it is fully indigenous and is always available at a reasonable price compared to foreign varieties. However, it is suggested that following the inoculation of sheep with these two vaccines, cellular immune responses be also evaluated and compared in the future researches.

Ethical Approval

All ethical standards were followed. The present study was carried out under the supervision of Ethics Committee for Research at Bu-Ali Sina University of Hamedan (ethical No. IR.BASU.REC.1398.017).

Conflicts of Interest Disclosure

On behalf of all authors, the corresponding author declares that there is no conflicts of interest.

Acknowledgments

The authors are grateful for financial supports of Bu-Ali Sina University of Hamadan and Razi Vaccine and Serum Research Institute.

References

1. Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. Croat Med J. 2010;51(4):296-305. doi: 10.3325/cmj.2010.51.296.
2. Hassani-TabaTabaei A, Firouzi R. Animal diseases due to bacteria. 2nd ed. Tehran University Press; 2005. p. 317-322. [Persian].
3. Garrity GM, Boone DR, Castenholz RW. Bergey's Manual of Systematic Bacteriology. 2nd ed. New York: Springer; 2001. p. 721.
4. Osterman B, Moriyon I. International Committee on Systematics of Prokaryotes; Subcommittee on the taxonomy of Brucella. Int J Syst Evol Microbiol. 2006;56(5):1173-5.
5. Dostdari S, Hassannya E, Khafri A, Emadi A, Bagherinejad

A, Alamyan S, et al. Stability Study of Rev1 Reduced dose Brucellosis Vaccine Produced by Razi Institute in Iran. Veterinary Researches & Biological Products. 2017;30(3):26-33. doi: 10.22092/vj.2017.109881. [Persian].
6. Jones LM, Entessar F, Ardalan A. Comparison of living vaccines in producing immunity against natural Brucella melitensis infection in sheep and goats in Iran. J Comp Pathol. 1964;74:17-30. doi: 10.1016/s0368-1742(64)80003-5.
7. Zoghi E. Researches about brucellosis. 1st ed. Agricultural and Natural Resources Research Organization; 1990. p. 66-7. [Persian].
8. Corbel MJ. Identification of the immunoglobulin class active in the Rose Bengal plate test for bovine brucellosis. J Hyg (Lond). 1972;70(4):779-95. doi: 10.1017/s0022172400022622.
9. Blasco JM, Estrada A, Mercadal M. A note on adult sheep vaccination with reduced dose of *Brucella melitensis* Rev 1. Ann Rech Vet. 1984;15(4):553-6.
10. Díaz-Aparicio E, Marín C, Alonso-Urmeneta B, Aragón V, Pérez-Ortiz S, Pardo M, et al. Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. J Clin Microbiol. 1994;32(5):1159-65.
11. Stournara A, Minas A, Bourtzi-Chatzopoulou E, Stack J, Koptopoulos G, Petridou E, et al. Assessment of serological response of young and adult sheep to conjunctival vaccination with Rev-1 vaccine by fluorescence polarization assay (FPA) and other serological tests for *B. melitensis*. Vet Microbiol. 2007;119(1):53-64. doi: 10.1016/j.vetmic.2006.08.004.
12. Shome R, Gupta VK, Narayana Rao K, Shome BR, Nagalingam M, Rahman H. Detection of *Brucella melitensis* Rev.1 vaccinal antibodies in sheep in India. Adv Anim Vet Sci. 2014;2(3S):19-22. doi: 10.14737/journal.aavs/2014/2.3s.19.22.
13. Benkirane A, Idrissi AH, Doumbia A, de Balogh K. Innocuity and immune response to *Brucella melitensis* Rev.1 vaccine in camels (*Camelus dromedarius*). Open Vet J. 2014;4(2):96-102.
14. Allan GS, Chappel RJ, Williamson P, McNaught DJ. A quantitative comparison of the sensitivity of serological test for bovine brucellosis to different antibody classes. J Hyg (Lond). 1976;76(2):287-98. doi: 10.1017/s0022172400055182.
15. Gharib Mambini E, Gharib Mambini M, Moradi Garavand M, Rezaie A, Mashkuh M, Kenarkuhi M, et al. Survey on immunogenicity efficacy of a dual vaccine, combined Rev.1 and sheep pox vaccine. J Vet Clin Res. 2014;5(1):43-50. [Persian].
16. Aldomy F, Alkhawaldeh M, Younis IB. Immune responses of goats (Shami breed) to vaccination with a full, reduced and conjunctival dose of brucevac (*Brucella melitensis* Rev.1) vaccine. Pak Vet J. 2009;29(4):149-53.