ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2022; 8(3): 05-08
www.allresearchjournal.com
Received: 04-01-2022
Accepted: 06-02-2022

Khabibulo Khamdamov
Senior Researcher, Brucellosis Research Laboratory at the Veterinary Research Institute, Samarkand, Uzbekistan

Feruza Khudoyorova
Senior Researcher, Brucellosis Research Laboratory at the Veterinary Research Institute, Samarkand, Uzbekistan

Murod Avliyoqulov
Senior Researcher, Brucellosis Research Laboratory at the Veterinary Research Institute, Samarkand, Uzbekistan

Corresponding Author:
Khabibulo Khamdamov
Senior Researcher, Brucellosis Research Laboratory at the Veterinary Research Institute, Samarkand, Uzbekistan

Application of reduced doses of brucellosis vaccine on small ruminants

Khabibulo Khamdamov, Feruza Khudoyorova and Murod Avliyoqulov

Abstract
Currently two vaccines from strains *B. abortus* 19 and *B. melitensis* Rev 1 are widely used for immune prophylactic of animal brucellosis. Using of strain Rev 1 vaccine is restricted due to its reactogenicity, frequent abortions and long time persistence of agglutinins in blood serum (up to 3-5 years) that result in false positive tests during serological diagnostics. Vaccine from *B. abortus* strain 19 is not reactogenis and does not cause abortions. On small ruminants this vaccine is used by full doses (40 bn microbe bodies, m.b.) on the following scheme: at 3-5-month age, before insemination and further each year in the same doses. However, in so doing this vaccine stimulate high agglutinins titers in blood that impede conducting of reliable diagnostics and cause organism sensitization. It was established that application of reduced dose of the vaccine *B. abortus* strain 19 against the background of full dose vaccination enables to avoid long time agglutinin persistence in serum of small ruminants and provide the same immunity strength as in full dose vaccinated. This make it possible to save considerable amount of means spend for vaccines purchase and to avoid undesirable effects emerging in using of full doses on small ruminants.

Keywords: diagnostics, brucellosis, vaccine, antibody, sensitivity, agglutinins, vaccination

Introduction

Significance of the problem

Brucellosis is the most common zoonosis in humans. Transmission of this disease occurs mainly by inhalation of infected aerosols, by animal contact, and by conjunctival and gastrointestinal routes. The gastrointestinal route is the most common portal of entry of Brucella in humans through ingestion of raw milk or its products and raw liver or meat. Transmission of *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis* in animals also occurs by ingestion of contaminated abortions, discharge materials, or contaminated pasture plants. In contrast, gastrointestinal transmission is not important under natural conditions for *B. ovis*, where the sexual route seems to be the most probable route of infection. Most isolates of *B. ovis* are urease negative. Due to serious economic losses and public health risk, extensive efforts have been conducted to prevent the disease in animals through vaccination programs. Live attenuated vaccines have been developed and successfully used worldwide against bovine brucellosis. Currently, no effective vaccines are available for the prevention of human brucellosis. *B. abortus* S19 live vaccine has been extensively used to prevent bovine brucellosis. The S19 vaccine strain was first isolated from the milk of a Jersey cow in 1923 and, while stored in the laboratory at room temperature, developed an attenuated phenotype. Numerous efficacy studies conducted with cattle for this vaccine have demonstrated that 70% of the vaccinated cattle are protected from a wild-type exposure. The effectiveness depended on a series of variables, including the age of the vaccinated animal, the prevalence of the disease in vaccinated herds, and the dose and route of the vaccination. Although S19 typically exhibits low virulence in cattle, the vaccine can cause abortions when administered to pregnant animals at rates between 1 to 2.5%. A less-frequent adverse effect of S19 vaccination is the development of an arthropathy associated with *Brucella* antigen-containing immune complexes.

Worldwide studies on the issue

Most of the researchers assured that the use of test& slaughter approach can be effective if herd brucellosis prevalence is not higher than 2%.

At higher prevalence level the spread intensity surpasses modern capabilities of on-time diagnosis and remove of infected animals. That's why in regions with high brucellosis prevalence vaccination is the main mean of struggle with brucellosis. Many years scientists from different countries are try to develop and improve means of active immunization of animals against brucellosis. The ideal vaccine should possesses life time immunity, does not stimulate producing of agglutinins in blood (or at least for a long time), with simple application and cheap cost and should be safe for human and not shedding to environment.

So far it was not possible to develop such bio preparation. Considering that in brucellosis, as in many other infections, the immunity is relative by strength and length it should be noted that the creation of ideal vaccine is almost impossible.

Brucellosis vaccination does not provide an absolute immunity. Irrespective of kind of vaccine and immunization methods the immunity can be disrupted by massive dose of pathogen. But the use of sufficiently immunogenic vaccine (for example S19 vaccine) considerably reduces clinical manifestation of disease (such as abortions and dead offspring) that results in decrease of infection spread because during abortions occurs the most intensive brucella shedding. The use of this vaccine in Soviet countries was started in 1952 for calves vaccination and since 1955 it's used for adult cattle immunization. In cattle herds vaccinated by S19 were observed stopping abortions and considerable decrease of disease prevalence. Vaccinated calves are becoming strongly resistant to exposure by virulent strain till 19 months. The mass vaccination does not only reduce the rate of abortions but in some herds totally puts an end to it. For example, in not successful non vaccinated herds only 24% of cows delivered normal offspring whereas in vaccinated herds 99% delivered healthy calves.

Different ways of active young and adult cattle immunization by S19 vaccine have been developing in many countries. As the number of researches shows the problem of high agglutinins titers after application of standard dose S19 subcutaneously can be solved by using small doses of this vaccine. According to many authors the immunity strength is not lower than in use of standard dose. For example Finney, Barton and Lomme note that in use of S19 in doses 1-3 x 109 CFU instead of standard dose 25-125 x 109 CFU the protection level does not decrease, vaccination of cows at different gestation periods is safe and serological testing of cattle vaccinated at maturity age becoming possible.

The purpose of the researches

For small ruminants vaccination in Uzbekistan have been used vaccine Rev-1 *B. melitensis*. But the use of this vaccine is restricted due to high reactogenicity, abortogenicity and long time agglutinins persistence (up to 3-5 years). That's why in many regions S19 is used for vaccination of small ruminants, because this vaccine is neither reactogenic nor abortogenic. Young animals vaccinated at 3-5 months age by 40 x 109 CFU subcutaneously and further revaccinated before insemination and each year by the same dose. The main shortcoming of vaccination by this strain in such dose is long time agglutinins persistence that does not allow to differentiate naturally infected animals from vaccinated. For this reason we pursued the development of a vaccination method for small ruminants that would not stimulate long-time persistence of agglutinins in blood. For this purpose we have tested immunogenic characteristics of the *Brucella* strain 19 in applications of reduced doses on small ruminants against the background of full dose vaccination.

Materials and Methods

There were 25 lambs used in experiments, divided to 5 groups. All of them were negative by RBT and TAT. Four groups were vaccinated by S19 subcutaneously at 4 month age, whereas 5th group wasn't and served as a control. After a year since first vaccination four groups were revaccinated subcutaneously by the following doses: 1st group – 500 mln of m.b.. second by 1 bn, third by 2 bn and fourth group by full dose 40 bn. 10 month later all animals were infected by standard virulent strain *B. melitensis* Novocherkassk-102. Then on 40th day after the exposure internal organs of all animals were tested to isolate brucella.

Table 1: Experimental design

Group #	Number of animals	First vaccination: full dose	Revaccination by different doses *X* *10^9 CFU	Infection by *B. melitensis* N-102 10 months later	Bacteriologic analysis 40 days later
1	5	5	0,5	5	5
2	5	5	1,0	5	5
3	5	5	2,0	5	5
4	5	5	40	5	5
5 (control)	5	-	-	5	5
Total	*25*	*20*	*20*	*25*	*25*

Results

For studying the dynamics of serological changes the blood of animals had been checked periodically. After first vaccination by full dose blood was checked on 5th, 15th, 30th day and then each month. First serological changes were observed on 15th day after vaccination. High agglutination titers appear by 30th day (1:100 and 1:200) and fall down at 330-360th day (1:50).

Table 2: Dynamic of agglutinins titers after first full dose vaccination (average of group)

Group #	15 days	30 days	90 days	150 days	210 days	270 days	330 days	360 days
1	70 (50-100)	120 (100-200)	200 (all)	160 (100-200)	100 (all)	90 (50-100)	70 (50-100)	55 (25-50)
2	70 (50-100)	120 (100-200)	200 (all)	180 (100-200)	90 (50-100)	70 (50-100)	60 (50-100)	40 (25-50)
3	90 (50-100)	140 (100-200)	200 (all)	140 (100-200)	90 (50-100)	90 (50-100)	70 (50-100)	55 (25-50)
4	70 (50-100)	120 (100-200)	200 (all)	160 (100-200)	100 (all)	90 (50-100)	70 (50-100)	55 (25-50)
5	N	N	N	N	N	N	N	N

After revaccination by different doses the high agglutinins titers again appear by 30th day. These high titers were observed at animals vaccinated by full doses till 180th day, whereas at animals revaccinated by reduced doses these titers were 5 times lower by this time. By 210th day all animals revaccinated by reduced doses showed serological titers lower than diagnostic threshold. On the next two tests on 270th and 300th day all these animals were negative both by RBT and TAT. Animals of 4th group revaccinated by full doses were seropositive even on 300th day with titers higher than diagnostic threshold.

Table 3: Dynamic of agglutinins titers after revaccination by reduced (1-3 groups) and full doses (4th group), average of group

Group # and vaccine doses		15 days	30 days	60 days	120 days	180 days	240 days	270 days	300 days
1	$0.5x10^9$	10 (25)	110 (100-200)	120 (100-200)	100 (all)	30 (25-50)	5 (25)	N	N
2	$1 x10^9$	20 (25-50)	120 (100-200)	140 (100-200)	120 (100-200)	30 (25-50)	5 (25)	N	N
3	$2x10^9$	20 (50)	125 (25-200)	120 (100-200)	100 (all)	30 (25-50)	15 (25)	N	N
4	$40x10^9$	70 (50-100)	160 (100-200)	200 (all)	200 (all)	160 (100-200)	90 (50-100)	80 (50-100)	75 (25-100)
5	n/vacc	N	N	N	N	N	N	N	N

10 month later after second vaccination all animals were infected by standard virulent strain *B. melitensis* Novocherkassk-102 by 625 thousands of m.b. subcutaneously. On 40th day after the exposure samples from heart, liver, spleen, kidney, marrow and lymph nodes of all animals were tested for brucella presence. Bacteriologic tests revealed brucella cultures from 2 animals in each 1 and 2nd groups that testifying on 60% of immunity. Amongst animals of 3rd and 4th groups only one animal of each group proved to be ill with brucellosis that means that the animals in these group have 80% immunity. And finally from all the animals of 5th had been isolated virulent strain *B. melitensis* Novocherkassk-102 – in total 30 brucella cultures that means 100% were infected.

Table 4: Immunity level: bacteriological study on 40th day after expose by *B. melitensis* standard strain "Novocherkassk-102"

Group # and vaccine doses		Number of animals in group	Number of animals from which isolated brucella cultures	Number of isolated cultures	Immunity level, %
1	$0.5x10^9$	5	2	5 + 6	60
2	$1 x10^9$	5	2	4+7	60
3	$2x10^9$	5	1	3	80
4	$40x10^9$	5	1	3	80
5	n/vacc	5	5	30	0

Thus it was established that the application of a reduced dose of the vaccine *B. abortus* strain 19 against the background of full dose vaccination provided the same immunity as full dose vaccination: the immunity of animals to brucellosis vaccinated by the reduced dose 2×10^9 CFU was not weaker than the immunity of animals vaccinated by full dose 40×10^9 CFU. This reduced dose also enabled the avoidance of long time agglutinin persistence in the serum of small ruminants. This will make it possible to reduce the cost of vaccines purchases as well as avoid undesirable effects emerging as seen in the use of full doses on small ruminants.

Discussions

The results of this study confirm that currently available serologic surveillance tests do not detect seroconversion following *B. abortus* 19 vaccination in sheep when applied in reduced doses. In many European countries, including Italy, vaccination against brucellosis is not allowed. The eradication programs for bovine and ovine-caprine brucellosis have centered on the serologic screening of herds to detect infected animals and on a surveillance system on the vaccine's use. In this situation, the limitations of standard serologic tests in identifying vaccinated animals are emphasized. Several authors have proposed the use of antigenic components different from LPS as a means to improve the diagnosis of bovine brucellosis. As far as we know, however, only two studies have been performed to measure the humoral immune response of vaccinated sheep against cytoplasmic proteins of *Brucella*. In those studies, no antibody response to reduced doses *B. abortus* S19 was detected, indicating that this protein is not a suitable antigen for differentiating vaccinated from infected cattle. Limet et al. suggested that antigens not present in S19 should be used to differentiate vaccinated from infected animals. Another possibility, however, is that because of transient exposure to *Brucella* antigens in S19-vaccinated sheep, poorly immunogenic components fail to significantly impact the immune system. We have previously shown that the 18-kDa cytoplasmic protein is present in all *Brucella* species, including S19. As shown here, although vaccinated animals developed a slight IgG response against cytoplasmic proteins of *Brucella*, their levels were much lower than those found in infected animals. The marked difference in antiprotein IgG levels between vaccinated and infected cattle is probably explained by differences in the duration of the exposure to *Brucella* antigens. In the case of infection with virulent strains, the exposure to *Brucella* antigens persists for longer periods, producing a stronger antibody response. This fact indicates the potential usefulness of protein antigens for diagnosing active brucellosis. The present results confirm our preliminary findings, since vaccinated animals developed a transient anti-18-kDa protein response that was much lower than that of infected animals.

Conclusions

It was established that the application of a reduced dose of the vaccine *B. abortus* strain 19 against the background of full dose vaccination provided the same immunity as full dose vaccination: the immunity of animals to brucellosis vaccinated by the reduced dose 2×10^9 CFU was not weaker than the immunity of animals vaccinated by full dose 40×10^9 CFU. This reduced dose also enabled the avoidance of long

time agglutinin persistence in the serum of small ruminants. This will make it possible to reduce the cost of vaccines purchases as well as avoid undesirable effects emerging as seen in the use of full doses on small ruminants.

References

1. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory. Bacteriological methods: Institut National de la Recherche Agronomique, Paris, France, 1988, 13-61.
2. Chappel RJ, McNaught DJ, Bourke JA, Allan GS. Comparison of the results of some serological tests for bovine brucellosis. J Hyg. 1978;80:365-371.
3. Cheville NF, Stevens MG, Jensen AE, Tatum FM, Halling SM. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus*. Am. J Vet. Res. 1993;54:1591-1597.
4. Cloeckaert A, Kerkhofs P, Limet JN. Antibody response to *Brucella* outer membrane proteins in bovine brucellosis: immunoblot analysis and competitive enzyme-linked immunosorbent assay using monoclonal antibodies. J Clin. Microbiol. 1992;30:3168–3174.
5. Blasco JM. A critical step for decision makers: control or eradication of *Brucella melitensis*? In *Brucella melitensis* in Eurasia and the Middle East. FAO technical meeting in collaboration with WHO and the OIE, May, Rome. Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Rome, 2009.
6. Murray PR, Corbel M. *Brucella*. In Topley and Wilson's microbiology and microbial infections: bacteriology (S.P. Boriello, P.R. Murray & G. Funke, eds), 10th Ed. Edward Arnold, London, 2007, 1719-1751.
7. Palmer DA, Douglas JT. Analysis of *Brucella* lipopolysaccharide with specific and cross-reacting monoclonal antibodies. J clin. Microbiol. 1989;27(10):2331-2337.
8. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet infect. Dis. 2006;6:91-99.
9. Refai M. Incidence and control of brucellosis in the Near East region. Vet. Microbiol. 2002;90:81-110.
10. Robinson A. *Brucella melitensis* infections: foodborne versus animal contact. In *Brucella melitensis* in Eurasia and the Middle East. FAO technical meeting in collaboration with WHO and the OIE, May, Rome. Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Rome, 2009.
11. World Health Organization (WHO). WHO Technical Report Series No. 68. Sixth report of the WHO Expert Committee on Biological Standardization. WHO, Geneva, 1953. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_68. pdf.
12. Ruppanner R, Meyer ME, Willeberg P, Behymer DE. Comparison of the enzyme linked immunosorbent assay with other tests for brucellosis, using sera from experimentally infected heifers. Am. J Vet. Res. 1980;41:1329-1332.
13. Saegerman C, Weynants V, Vo TK, Dewaele L, Tibor A, Denoel P, *et al*. Evaluation de l'activite´ protectrice de la fraction de paroi de *Brucella* insoluble dans le SDS et identification d'antige`nes de *Brucella* utilisables por le diagnostic, p. 221–233. In AUPELF-UREF (ed.), Biotechnologies du diagnostic et de la pre´vention des maladies animales. John Libbey Eurotext, Paris, 1994.
14. Stevens MG, Tabatabai LB, Olsen SC, Cheville NF. Immune responses to superoxide dismutase and synthetic peptides of superoxide dismutase in cattle vaccinated with *Brucella abortus* strain 19 or RB51. Vet. Microbiol. 1994;41:383-389.
15. Sutherland SS, Den Hollander L. Comparison of an enzymelinked immunosorbent assay using monoclonal antibodies and a complement fixation test for cattle vaccinated and infected with *Brucella abortus*. Vet. Microbiol. 1986;12:55-64.
16. World Organisation for Animal Health (OIE). Chapter 2.7.2.: Caprine and ovine brucellosis (excluding *Brucella ovis* infection). In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 6th Ed. OIE, Paris, 2008, 974–982.
17. World Organisation for Animal Health (OIE). OIE Guide 3: International reference antibody standards for antibody assays. In OIE quality standard and guidelines for veterinary laboratories: infectious diseases, 2nd Ed. OIE, Paris, 2008, 45-52.
18. World Organisation for Animal Health (OIE). Chapter 14.1.: Caprine and ovine brucellosis (excluding *Brucella ovis*). In OIE Terrestrial Animal Health Code, 18th Ed. OIE, Paris, 2009, 679-684.