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Identification of the Excretion of *Brucella Melitensis* Vaccine Strain Rev.1 in Lactating Ewes and the Assessment of Antibody Response in their Lambs

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ABSTRACT

Background: Brucellosis is a zoonotic disease and humans get the infection mostly through consumption of raw milk. Vaccination is the best way to control brucellosis and Iran uses Rev.1 vaccine in sheep and goat flocks. It is evident that the vaccine may shed through milk so it can infect humans. The objective of the present study is to assess the shedding of the vaccine in lactating ewes and its possible immunity in their lambs through milk feeding.

Methods: In a two-month period post-parturition, reduced dose Rev.1 vaccine was injected to 50 parturited ewes. From the first day of vaccination, mixed milk samples were collected and continued for 2 months. Then the samples were tested by PCR method and the sera from 70 lambs of the examined ewes were tested by modified Rose Bengal test while they were feeding milk.

Results: From the 6th day until the 27th day post-vaccination, PCR represented the DNA of Rev.1 in the milk samples. All the lamb's sera were negative in the serological test.

Conclusion: As the presence of Rev.1 in milk was confirmed in this study, it is important to consider the role of the vaccine strain as a risk of infection in humans. Moreover, as the serological response in the lambs was negative, it seems that the vaccine strain didn't immunize the lambs through milk feeding so the vaccination of lambs is necessary in small ruminant's flocks.

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Introduction

Brucella melitensis is a gram negative facultative anaerobic bacterium which is a major cause of small ruminant's abortion in many small ruminant raising countries worldwide (1). Abortion along with complications such as decrease in milk production and infertility, cause economic losses to this industry. In male animals, the symptom of the infection is orchitis which may result in the exclusion of affected animals (2).

Brucellosis is a zoonotic disease and constitutes a serious hazard for public health. *B. melitensis* annually infects more than 500000 humans in Iran (3). Humans get the infection through consumption of raw milk, or other dairy products, inhalation of infected aerosols and direct contact with aborted materials of infected animals (4). No vaccine is available for human use so the key of prevention of human brucellosis is controlling the disease in animals especially in small ruminants (5).

Control of small ruminant's brucellosis in Iran started in 1963 and since then various programs have been stablished which have resulted in considerable reduction in the rate of brucellosis in both humans and animals (3, 6). In heavily infected countries vaccination is the best way to control the disease (7, 8). So the policy of Iran for control of brucellosis is utilization of Rev.1 vaccine in sheep and goat flocks (3). Rev.1 vaccine is an attenuated strain of *B. melitensis* which is administrated via subcutaneous or conjunctival route and induces suitable protection against abortion (8, 9). Lambs older than 3 months of age and before mating are vaccinated by full dose Rev.1 vaccine and vaccination of adult animals are conducted with reduced dose of the vaccine (10).

Since vaccination of ruminant population in Iran began, Brucellosis has dramatically decreased among human population (6). However, the vaccine has some disadvantages. It interferes with serological tests, can infect humans, may cause abortion in pregnant animals, and does not always protect animals in the field. In addition, like the wild strain, the vaccine may shed through milk and vagina, so it can spread horizontally (11, 12) and may cause infection in humans (13). There is a report in south Africa in which the vaccine strain had infected human through horizontal transmission from vaccinated sheep (14).

Attempts for improving safety of Rev. 1 such as using a reduced dose vaccine and administration of the vaccine via conjunctival route had a limited effect on these adverse complications. The vaccine strain may excrete in milk even after conjunctival immunization (15).

There is a lack of data about the presence of Rev.1 vaccine in milk and its hazard for humans in our country. Moreover, immunity of lambs via feeding milk of vaccinated ewes is not clear. So the objective of the present study is to assess the shedding of the vaccine in the milk of lactating ewes and the presence of antibody response for the possible immunization in their lambs by feeding milk

Materials and Methods

Sampling and Vaccination procedure

In an industrial sheep flock, a total of 25 Roman breed and 25 sheep of other breeds including Lacaune, Charollais, Blanche du massif central, Il de France, and Suffolk were chosen for this experiment.

In a two-month period post-parturition, *Brucella melitensis* reduced dose Rev.1 vaccine (produced by Razi Vaccine and serum research Institute), with $0.5\text{-}3\times10^6$ live agents in a volume of 1 ml was injected subcutaneously to the selected animals. Mixed milk samples of the animals were aseptically collected two days after vaccination, and continued for 2 months. Totally, eleven samples were taken in this period. Then the samples were prepared for the molecular examination. Sera were collected from 70 lambs of the experimental ewes before feeding milk and then 2 months after vaccination of ewes, during a

two-months period sera samples were collected for the second time while the lambs were feeding milk. Then all the sera were examined using modified Rose Bengal test before and after feeding milk.

Polymerase Chain Reaction (PCR)

DNA was extracted from the milk samples using CinnaGen DNA extraction kit according to the manufacturer's instruction. Then PCR was conducted based on the method of Bricker and Halling, 1994 (16).

The primers amplified a 731-bp fragment of the Bmel gene. Forward and reverse primers included TGCCGATCACTTAAGGGCCTTCAT and AAATCGCGTCCTTGCTGGTCTGA respectively.

PCR was performed using 0.5 µM of each primer, 200 µM of each dNTPs, 2 mM $MgCl_2$ and 0.05 U Taq DNA polymerase. The final volume of reaction mixture was amounted to 25 µl including 24 µl master mix and 1 µl template DNA.

Amplification was carried out in the automated DNA thermal cycle using the following cycling parameters: Denaturation at 95 °C for 5 min, subsequently 30 cycles of 95 °C for 30s and 64 °C for 60s and 72°C for 60s. The final extension was performed at 72 °C for 5 min.

Modified Rose Bengal test

Sera and Rose Bengal antigen (Razi Vaccine and serum research Institute) were brought to room temperature. A volume of 75µ of each serum was placed on a white plastic plate. Then 25 µ of Rose Bengal antigen was added near the serum. Serum and antigen were mixed and were shaken for 4 minutes. Then the results were immediately read for agglutination.

Results

The vaccine strain wasn't detected in the milk samples until the 6th day post-vaccination. From the 6th day until the 27th day post-vaccination, PCR represented the DNA of Rev.1 in the milk samples. After this time, the vaccine wasn't detected during the study period.

The sera of the lambs which had been collected before feeding milk were negative in the serological test. Two months after vaccination of the ewes, the results of all the lamb's sera were also negative in the Modified Rose Bengal test.

Discussion

The present study revealed that Rev.1 vaccine excretes from milk after parturition of vaccinated ewes for a relatively long period. We detected Rev.1 between 6-27 days post-vaccination and after that, the mix samples were negative in the molecular test. It is evident that after vaccination with Rev.1, there would be a bacteremia at the first day and remains until 60 days post-vaccination. At the second week, the presence of vaccine strain reaches to its maximum level so the vaccine could be a risk for human (17). Elberg and Meyer (1959) showed that tissues of vaccinated animals clear the vaccine by 14 weeks after subcutaneous vaccination and using conjunctival route may diminish this period (18).

In the study of Ponsart et al. (2019) Rev.1 vaccine was administrated via conjunctival route to female and male goats and ibex. Then they evaluated the presence of vaccine at 0, 20, 45, 68 and 90 days post-vaccination using bacteriological methods. The experimental animals were euthanized and the Rev.1 strain was found in the organs such as urogenital swabs of the vaccinated goats and ibex. The researchers concluded that the highest risk of Rev.1 shedding is between day 20 and day 68. Moreover, shedding of the vaccine strain couldn't be rule out for longer period as it was detected at day 90 post-vaccination. Rev.1 was also detected in male animals which showed urogenital excretion at 20 or 45 days post-vaccination (15).

Alamian et al., (2015) didn't find Rev.1 strain in milk and vagina of Iranian fat-tailed ewes after parturition. These researchers used conjunctival

route for vaccine administration and moreover, they had just monitored the shedding of the vaccine until 24-hour post-parturition (19) which wasn't enough time to evaluate the vaccine excretion. In the current study, we didn't detect Rev.1 until 6th day post-vaccination too, while after this time, Rev.1 continued to be shed for 22 days.

In a study in 2008, in 4% of samples belonged to aborted cattle, Rev.1 vaccine was detected which showed that the vaccinated ewes which were kept in close contact with cattle, had shed and transmitted the strain horizontally to the cattle (20). Even using a reduced dose vaccine, as we had used in the present study, couldn't prevent the excretion of Rev.1 in milk. Nevertheless, El Idrissi et al. (2001) showed the reduced dose of Rev.1 decreases the vaccine shedding in milk (21).

In Iran, pregnant ewes and does which don't receive Rev.1 before pregnancy, are vaccinated after delivery. Our results showed these animals shed the vaccine strain through milk and people especially in rural areas consider the milk safe and consume it without proper heating. So they may be infected by Rev.1 which its symptoms are similar to brucellosis caused by the wild strain (22). In addition, as Rev.1 is resistant to streptomycin which is the choice antibiotic against the wild strain (23), the treatment of people who are infected by the vaccine strain is difficult. According to our results, it is important to consider the role of vaccine strain as a risk of infection in humans who use unpasteurized milk and other dairy products (24).

Conclusion

The present study showed that despite excretion of the vaccine strain via milk in 10 of 11 times sampling, the lambs didn't show antibody response against *B.melitensis* by feeding the milk. Although oral immunization reduced antibody response as all the lamb's sera in the current experiment didn't show positive reaction even in modified Rose Bengal test which has high sensitivity, vaccination of young animals is strictly recommended.

Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare no competing financial interest.

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