

REV-1 conjunctival vaccine administration in Turkey

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Summary

Brucellosis in sheep and goats has a major economic and zoonotic importance, and implementation of strategies for its control and eradication is essential in endemic areas. In this study, the enhanced abortion cases in small ruminants after conjunctival Rev-1 vaccine administration was examined by PCR in terms of probability of vaccine induced abort cases. Of the examined 77 cotyledons from the aborted fetuses belonging to 68 sheep and 9 goats, 70 (90.90%) were found to harbour *Brucella* spp. DNA. In the first, second and third trimester of gestation, the rate of 100%, 40% and 42.85% positive results were determined from the cotyledons of the small ruminants, respectively. In conclusion, the conjunctival route of Rev-1 vaccine administration was safe during field vaccination, compatible with the serological tests and induces less abortion compared with the subcutaneous route as long as the period of pregnancy is taken into consideration during the vaccination period.

Keywords: *Brucella* Rev-1 conjunctival vaccine, PCR, sheep, goat

Sheep and goat brucellosis caused by *Brucella melitensis* (*B. melitensis*) is a zoonotic disease. It is widespread in many areas of the world, and an especially a serious problem in developing countries because of their socio-economic situation (27, 28). Several serological studies have shown that brucellosis is still endemic in the livestock population of sheep and goats in the world (5, 9, 15, 16, 20, 23, 24) and in Turkey (2, 4, 17, 28).

In endemic areas where the levels of seroprevalence are high, vaccination is the preferred means for the control of brucellosis, whereas the test-and-slaughter policy may eradicate the disease without vaccination where prevalence levels are low (27). Interference with serological tests and increased abortion cases after vaccine administrations have been reported to be the limitations of both Rev-1 and reduced-dose Rev-1 vaccines administered via subcutaneous route. However, both problems were reportedly lessened by the administration of the Rev-1 conjunctival vaccine (11). Nevertheless, when mass vaccination is the only means of controlling the disease, a vaccination campaign should be recommended using the standard dose of Rev-1 administered by the conjunctival route when the animals are not pregnant or during the late lambing/kidding and pre-breeding season (12, 18). Considering the baseline

level of infection, the essential and appropriate control strategy of brucellosis in Turkey is vaccination (19, 27).

This study was aimed at examining cotyledon samples collected from aborted fetuses by PCR according to information obtained from farmers regarding the increase in abortion cases in sheep and goats after Rev-1 conjunctival vaccine administration.

Material and methods

Sample collection. The samples were collected between January 2013 and December 2014. In total, 77 aborted 33 sheep (n = 68) and goats (n = 9) barned in the Bursa city center and surrounding districts were examined for the presence of *Brucella* spp. DNA by PCR. Information about the pregnancy period of the aborted animals was obtained from the owners and recorded. Twenty-gram samples of placental cotyledon were taken from each of the aborted fetuses of sheep and goats. During sample collection, sterile latex gloves were used to prevent cross contamination. All samples were transferred immediately to the laboratory under cold chain. Detailed information about the samples is presented in Table 1.

DNA extraction. DNA was extracted from the placental cotyledons and with a commercial DNA extraction Kit (Qiagen) as described by the manufacturer. The genomic DNAs were stored at -20°C until use as template in PCR.

Positive control DNA. DNA extracted from *Brucella melitensis* (ATCC 23456) was used as a positive control in the study.

Primers. As described previously, primers consisted of the following sequences: F4; TCG AGC GCC CGC AAG GGG and R2; AAC CAT AGT GTC TCC ACT AA (22).

PCR reaction. Each reaction had a volume of 25 µl, including 22 µl reaction mixture containing 2.5 µl 10 × PCR buffer (without MgCl₂), 0.5 µl dNTP (10 mM), 1.5 µl MgCl₂ (25 mM), a 1 µl of each primer (10 pmol/µl), 0.25 µl Taq DNA polymerase (5 U/µl) (Fermantas, Vilnius, Lithuania), 17.25 µl deionized water and 1 µl template. Cycling parameters were as follows: initial denaturation at 95°C for 2 min followed by 35 cycles of denaturation at 94°C for 30 sec, 55°C for 1 min, extension 72°C for 30 sec and final extension 72°C for 10 min.

Agarose gel electrophoresis. PCR products were electrophoresed on a 1.5% agarose gel in TBE buffer (Thermo Scientific, Vilnius, Lithuania) containing 0.5 µ/ml of ethidium bromide at 100 V for 45 min and visualized under UV light. The PCR product was determined to be 905 bp.

Results and discussion

Information about the pregnancy periods of the animals is given in Table 1. In the examined samples obtained from aborted fetuses, 90.90% (70/77) were found to be positive. In the first trimester of pregnancy in all districts, the samples collected were determined as positive with the rate of 100% (65 cotyledons of sheep and goats). In the second and third trimesters of pregnancy, at the ratios of 40% and 42.85% positive results were determined, respectively. While 2 out of 5 cotyledons were found to be PCR positive in the second trimester of the pregnancy, positive PCR results were detected in 3 out of 7 cotyledons collected from fetuses aborted in the third trimester of pregnancy. These results were given in Table 2.

Tab. 1. Number of collected samples of cotyledons from sheep and goats according to pregnancy time

Gestation Period	District/Species					
	İznik		Akçalar		Bursa (Centre)	
	Sheep	Goat	Sheep	Goat	Sheep	Goat
1/3	12	–	21	–	25	7
2/3	3	–	–	–	2	–
3/3	–	–	2	–	3	2
Total	15	–	23	–	30	9

Tab. 2. Number and percentages of PCR results according to pregnancy period

District	Gestation Period					
	1/3		2/3		3/3	
	PCR + (%)	PCR – (%)	PCR + (%)	PCR – (%)	PCR + (%)	PCR – (%)
İznik	12 (100)	0 (0.00)	2 (66.66)	1 (33.33)	0 (0.00)	0 (0.00)
Akçalar	21 (100)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (100)
Bursa (Centre)	32 (100)	0 (0.00)	0 (0.00)	2 (100)	3 (60.00)	2 (40.00)
Total	65 (100)	0 (0.00)	2 (40.00)	3 (60.00)	3 (42.85)	4 (57.14)

Brucellosis is endemic in all neighbouring countries of Turkey (1, 10, 13, 14, 17, 25). It is not surprising that brucellosis is endemic in Turkey, given its geographic location. To the best of our knowledge, *B. melitensis* was reported in Merino sheep at the Bandırma Merinos Farm in 1944 (8). According to the data from Republic of Turkey Ministry of Agriculture and Forestry, the prevalence of brucellosis in sheep was determined to be 1.26% in the first sero-survey study conducted in 1989 in Turkey. In an other survey conducted in 2001, the individual and herd prevalence of brucellosis were declared to be 4.7% and 30%, respectively (26).

Given the experience of *B. melitensis* vaccine administration in small ruminants, the subcutaneous Rev-1 vaccine was used first, which produced a strong and long-lasting immunity. However, it had some drawbacks such as long-lasting serological response and vaccine induced abortions (3, 7, 8). Hence veterinarians were required to administer a reduced dose Rev-1 *B. melitensis* vaccine via a subcutaneous route at the end of pregnancy and during lactation in order to minimize the risk of abortion and increase the degree of safety (6). Yet determining whether the serological reaction was caused by the vaccine or infection was difficult. Thus, test-slaughter eradication programmes could not be applied effectively (6). Subsequently, Rev-1 vaccine was administered by the conjunctival route ($0.5-2 \times 10^9$ CFU) in young replacement animals all over the world, owing to a similar degree of safety as the subcutaneous route. Furthermore, the conjunctival route greatly reduced the serological response, making it consistent with eradication programs focused on test and slaughter concepts (12, 18). Turkey has adopted the same vaccine administration flow as in the past.

Turkey determined its policies for disease control and eradication based on the prevalence and distribution of brucellosis, as well as the capacity of veterinary services to administer the vaccine and track epidemiological parameters. Combating Brucellosis was carried out in accordance with the *Regulation on Combating Brucellosis* (Official Gazette dated 3.4.2009 and numbered 27189), the *Circular on Control and Eradication of Brucella with Conjunctival Vaccine* (dated 13.01.2012 and numbered 2012/03), and the annual *Circular on Fight Against and Control of Animal Movements*.

According to the National *Brucella* Surveillance Report for Ruminants, the prevalence of small ruminant herds and individuals in Turkey was 30 percent and 4.7 percent, respectively, in 2011 (26). *Brucella* Control and Eradication Project by Conjunctival Vaccination had been started in 2012 for 6 years in small ruminants. *B. melitensis* Rev-1 conjunctival vaccine was administered to all female sheep, goats, and breeding male sheep and goats in 2012 as part of a mass vaccination strategy in sheep and goats, with the exception of those

vaccinated with young Rev-1 subcutaneous vaccine. Adult female animals and breeding male animals that had remained unvaccinated from the previous year were decided to be vaccinated the following year, in addition to 3-6 month female lambs and kids. A six-year vaccination program was planned in this concept (21). In 2013, however, entire groups of sheep and goats were vaccinated regardless of pregnancy period or age.

In the study, samples were obtained between January 2013 and December 2014, which coincided with the start of mass vaccination, regardless of the sheep's gestation period the farms informed us about the increased abortion cases after a mass vaccination. At this time we collected the cotyledons of the aborted fetuses as described in material methods section and then they were examined for the presence of *Brucella* spp. *Brucella* DNA was found in all the cotyledons collected from the cases belonging to pregnant sheep and goats within first trimester of pregnancy by PCR. Despite the fact that conjunctival vaccination (reduced Rev-1 dose) resulted in fewer abortions than subcutaneous vaccination (6, 29), abortion cases were observed after vaccination, indicating that it may be a vaccine-induced issue. Since the research duration coincided with the vaccination period regardless of gestation period, it was a foregone conclusion that the positive results observed in our study will be higher in the first trimester of pregnancy.

When comparing cases in the 2nd and 3rd trimesters of pregnancy to those in the 1st trimester, we found that the number of aborted cases in the 1st trimester thought to be caused by *B. melitensis* Rev-1 conjunctival vaccine administration was very low. Compatible with our assumption, Bagues et al. (6) confirmed that the majority of vaccine-induced abortions occurred 40 to 60 days after vaccination, and that the percentage of abortions was significantly higher when sheep were vaccinated during the first two months of pregnancy than when they were vaccinated in the last month of pregnancy.

Consequently, it may be stated ignoring the gestation period during vaccine administration leads to an increase in abortion cases, especially in the first trimester of pregnancy, similar to the situation in Turkey in 2013. Although conjunctival vaccine administration provides a similar level of safety to subcutaneous vaccine administration, it also significantly reduces the serological response, which is consistent with eradication. In mass vaccine administrations, taking the pregnancy period into consideration assures safe, effective and efficient preventive control measures in terms of *Brucella* Control Programs. Therefore, working with farmers is critical to achieving success with mass (whole-flock) vaccination and obtaining information on individual flock members, such as pregnancy periods. In addition to vaccination, organisation of veterinary services, the strict control of animal movements, compulsory provision of adequate economic compensation of farmers should not be neglected when sustaining eradication programs.

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