

IMMUNE RESPONSES OF GOATS (SHAMI BREED) TO VACCINATION WITH A FULL, REDUCED AND CONJUNCTIVAL DOSE OF BRUCEVAC (*BRUCELLA MELITENSIS* REV.1) VACCINE

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ABSTRACT

Three groups of Shami goats were randomly vaccinated with Brucevac (Rev. 1) vaccine. Group 1 was vaccinated subcutaneously with a full dose (1.54×10^9 organisms). Group 2 was vaccinated conjunctively with one eye drop (5.2×10^8 organisms), while Group 3 was injected subcutaneously with a reduced dose (7.1×10^5 organisms) of vaccine. Blood samples were collected before vaccination, two, four, eight, 15 and 24 weeks post vaccination. All samples were tested through CFT, ELISA, SAT and Rose Bengal plate test. All serological tests used detected a higher percentage of vaccinated female kids with a full dose than they did in other groups vaccinated with a reduced dose or with a conjunctival dose of Rev.1 vaccine. The overall results suggested that 100% of animals vaccinated with a conjunctival dose became positive to CFT at two, four, eight and 15 weeks post vaccination, and then the percentage of seropositive animals declined and became 20% at 24 weeks post inoculation. The conjunctival route of vaccination significantly reduced the intensity and duration of the post vaccination serological response, which makes the use of this vaccine compatible with brucellosis programmes, even when these are based on a test-and-slaughter policy. The overall results showed that Shami goats responded to Rev.1 vaccine in the expected way. The majority of animals were seropositive to the CFT by two weeks after vaccination with higher numbers of seropositive animals in the kids group vaccinated with a full dose of Rev.1 vaccine.

Key words: *Brucella melitensis*, vaccine, immune response, goats.

INTRODUCTION

Brucellosis is an important infectious disease of sheep and goats and is considered to be the most serious zoonotic disease for humans (Gul and Khan, 2007). *B. melitensis* is primarily responsible for brucellosis in sheep and goats (Enright, 1990). Brucellosis occurs in small ruminants in the Mediterranean and Middle Eastern countries, particularly Iran, and spreads eastward to southern regions of Russia, Mongolia and northern China (Castrucci and Cilli, 1991).

The majority of abortions in sheep and goats occurred in the last month of gestation. Infectious diseases such as brucellosis, campylobacteriosis, toxoplasmosis, chlamydiosis and Q fever usually cause abortion in late gestation, therefore, special attention should be paid towards looking for them. They can be of considerable economic importance to the sheep and goat industry (Aldomy *et al.*, 2009).

The serological tests commonly used for the diagnosis of *B. melitensis* infection are the Rose Bengal Plate test (RBPT), SAT and CFT (Alton, 1990). Recently, the ELISA has been employed for the diagnosis of brucellosis. Control measures are based on strict hygiene and vaccination of susceptible animals like sheep and goats. Vaccination is regarded as a measure for reducing the prevalence of the disease

eventually to a level where eradication by test and slaughter can be considered. Of the vaccines now used for immunizing small ruminants against *B. melitensis*, Rev.1 vaccine is generally preferred (FAO/WHO, 1986; OIE, 2004).

The Rev.1 vaccine is indicated to protect small ruminants against brucellosis and to protect females from abortion in regions where the disease occurs. Conjunctival vaccination is safer than subcutaneous vaccination but is not safe enough to be applied regardless of pregnancy status of animals (Blasco, 1997) and the duration of immunity conferred by this method of vaccination is the subject of controversy. This study was carried out to demonstrate the immune responses of kids and adult goats to Brucevac vaccine (full dose, reduced dose and conjunctival dose) by the use of the CFT, ELISA, SAT and Rose Bengal plate test.

MATERIALS AND METHODS

General procedure and origin of specimens

This study was conducted from November 2005 to May 2006 at a well-managed, feedlot operation in Alwala Goats Farm (Ministry of Agriculture Shami Goats' Station), Amman, Jordan. Experimental animals were sound and apparently healthy and were randomly

selected from a known source obtained from brucellosis free stocks and reared in isolation.

Three groups of ear tagged goats were selected randomly. Group 1 consisted of six female kids aged between 3 and 5 months, Group 2 consisted of eight female goats aged between 9 and 11 months and Group 3 contained 10 male goats aged 12-18 months. All animals were provided with adequate housing and kept under conditions in which they would remain healthy, would not be subjected to cross-infection, and should not be overcrowded. They were provided with good hygienic conditions, a balanced diet and abundant supply of water.

Vaccination

All animals were bled before vaccination and then were inoculated with Rev.1 vaccine prepared by the Jordan Bio-Industries Centre (JOVAC), Aman, Jordan. Each animal in Group 1 was vaccinated subcutaneously with a full dose (1.54×10^9 viable organisms, Batch No. BR 01016-01) of the Brucevac (Rev.1) vaccine; each animal in Group 2 was vaccinated conjunctively with one eye drop (5.2×10^8 viable organisms, Batch No. BR 5905-01) of the Brucevac (Rev.1) vaccine and each animal in Group 3 was injected subcutaneously with a reduced dose (7.1×10^5 viable organisms, Batch No. BR 4805-02) of Brucevac (Rev.1) vaccine. Blood samples were taken from animals of all groups before vaccination and then at 2, 4, 8, 15 and 24 weeks post vaccination for serum collection. All serum samples collected from the experimental animals were subjected to Rose Bengal Plate test (RBPT), CFT, SAT and ELISA.

Complement fixation test (CFT)

The hot CFT method for *Brucella melitensis* was used, as described by Alton *et al.* (1988). The CFT kit produced by the JOVAC, Amman, Jordan was used. As a general rule, the minimum positive titre of vaccinated animals is double the minimum positive titre of non-vaccinated animals (Staak, 1990). However, sera completely inhibiting lysis at a dilution of 1 in 10 were considered positive, partial lysis was taken as doubtful and complete lysis was regarded as negative at this dilution (Cox *et al.*, 1977).

Rose Bengal Plate test

Serum samples and Brucella antigen stained with Rose Bengal produced by JOVAC, Amman Jordan were brought to room temperature before testing. One drop (30 μ l) of undiluted test serum was placed on a white plate and one drop (30 μ l) of the antigen was placed beside it. Antigen and serum were mixed thoroughly with a disposable wooden stick and then the test plate was moved vigorously in a wide circle, five to six times clockwise, followed by five to six times anticlockwise. The plate was examined after four

minutes. Any degree of agglutination was considered to be positive.

Serum agglutination test (SAT)

The test was based on the Wright-test produced by JOVAC, Amman Jordan. SAT titers $\geq 1:40$ were indicative of Brucella antibodies.

ELISA

An indirect ELISA kit produced by JOVAC, Amman Jordan was used. Test result was generated through the following equation:

Percentage positivity (%p) = absorbance (test sample)

OD of sample - OD of negative control

-----X 100

OD of strong positive control - OD of negative control

OD= optical density.

Strong positive control antibody lies between >60-100.

Moderate positive control antibody lies between >31-59 and negative control antibody lies between 0-30.

RESULTS

In the present study, all animals which were bled before vaccination were negative for brucellosis. No significant local or systemic reaction was observed during the first three weeks of the study period.

Female kids vaccinated with a full dose

In six female kids vaccinated with live attenuated full dose (1.54×10^9 CFU) of Brucevac (Rev 1) vaccine, peak mean CFT titre (1:107) was observed two weeks, whereas peak mean SAT titre (1:96) was observed 15 weeks post vaccination. The titres then declined to 1:85, 1:36, 1:16 and 1:8 for the CFT at four, eight, 15 and 24 weeks post vaccination, whereas the titre of 1:80 was demonstrated eight and 24 weeks post vaccination for the SAT (Table 1).

Table 1: Mean CFT and SAT titres in female kids vaccinated with a full dose of Brucevac (Rev.1) live attenuated *B. melitensis* vaccine

Weeks post vaccination	CFT titres	SAT titres
0	1:0	1:0
2	1:107	1:37
4	1:85	1:70
8	1:36	1:80
15	1:16	1:96
24	1:8	1:80

The serological results also indicated that CFT detected antibodies to Brucella in 100, 100, 100, 100 and 50% of animals two, four, eight, 15, and 24 weeks after vaccination, respectively compared to 100, 100, 100, 100, 100%, for the ELISA and the RBPT. The SAT detected

the lowest percentages (83%) two weeks after vaccination but detected 100% four, eight, 15 and 24 weeks post vaccination, respectively. The RBPT and the ELISA test gave similar results in this group.

Goats vaccinated with a conjunctival dose

For adult female goats vaccinated with live attenuated conjunctival dose (5.2×10^8 organisms) of Brucevac (Rev.1) vaccine, peak mean CFT and SAT titres (1:87 and 1:120, respectively) were observed four weeks post vaccination. They then declined to 1:67, 1:32 and 1:6 for the CFT and 1:72, 1:24 and 1:14 for the SAT at eight, 15 and 24 weeks post vaccination, respectively (Table 2).

Table 2: Mean CFT and SAT titres in female goats vaccinated with a conjunctival dose of Brucevac (Rev.1) *B. melitensis* vaccine

Weeks post vaccination	CFT titres	SAT titres
0	1:0	1:0
2	1:63	1:33
4	1:87	1:120
8	1:67	1:72
15	1:32	1:24
24	1:6	1:14

The serological test results indicated that CFT detected antibodies to Brucella in 100, 100, 100, 100 and 20% of animals two, four, eight, 15, and 24 weeks after vaccination compared to 100, 100, 100, 100 and 100%, in the ELISA and the RBPT. Only 20% animals vaccinated with the conjunctival dose gave positive titre to CFT (1:10) 6 months post vaccination i.e. nearly sera of all vaccinated animals were negative to CFT 24 weeks after vaccination.

The SAT detected 63% of animals two weeks post vaccination and detected the lowest percentages (20 and 0%) 15 and 24 weeks post vaccination. The RBPT and the ELISA gave similar results in this group.

Male goats vaccinated with a reduced dose

In male goats vaccinated with live attenuated reduced dose (7.1×10^5 CFU) of Brucevac (Rev.1) vaccine; peak

mean CFT and SAT titres (1:38 and 1:68, respectively) were observed two weeks after vaccination. They then declined to 1:16, 1:11, 1:5 and 1:3 for the CFT and 1:33, 1:7, 1:0 and 1:0 for the SAT at 4, 8, 15 and 24 weeks post vaccination, respectively (Table 3).

Table 3: Mean CFT and SAT titres in bucks vaccinated with a reduced dose of *Brucella melitensis* Rev.1 vaccine

Weeks post vaccination	CFT titres	SAT titres
0	1:0	1:0
2	1:38	1:68
4	1:16	1:33
8	1:11	1:7
15	1:5	1:0
24	1:3	1:0

The percentage of vaccinated animals positive to CFT, SAT, ELISA and RBPT are presented in Table 4. The CFT detected antibodies to Brucella in 100, 100, 71, 0 and 0% of animals two, four, eight, 15, and 24 weeks after vaccination respectively compared to 100, 100, 100, 100, 40%, in the ELISA. The SAT detected the lowest percentages (88, 67, 0, 0 and 0%) two, four, eight, 15 and 24 weeks post vaccination. The RBPT and the ELISA gave similar results in this group.

DISCUSSION

Despite advances in molecular techniques, serology remains at the forefront of eradication and surveillance programmes for veterinary diseases and is an important tool in the fight against human brucellosis (McGiven, 2008). The immunological tests conducted on the three groups of goats vaccinated with Brucevac Rev.1 vaccine indicated that no previous exposure to Brucella had taken place in these animals.

In the light of the occurrence of brucellosis in sheep and goats in many countries (Gul and Khan, 2007), the most effective measure to reduce prevalence to a point where eradication by test-and-slaughter is feasible, is through an annual campaign to vaccinate adult sheep and

Table 4: Percentages of seropositive animals (Group 3) to serological tests post vaccination subcutaneously with a reduced dose of live attenuated *Brucella melitensis* Rev.1 vaccine

Weeks post vaccination	Total number of animals	Percentages of seropositive animals to RBPT	Percentages of seropositive animals to CFT	Percentages of seropositive animals to ELISA	Percentages of seropositive animals to SAT
0	10	0	0	0	0
2	8	100	100	100	88
4	9	100	100	100	67
8	7	100	71	100	0
15	8	100	0	100	0
24	5	40	0	40	0

goats with a reduced dose or with a conjunctival dose of Rev 1 vaccine and vaccination of lamb and kids 3-8 months old with a full dose of Rev.1 vaccine. *Brucella melitensis* strain Rev. 1 is recognised all over the world as the reference vaccine for protecting sheep and goats against *B. melitensis* infection (Verger, 1995; OIE, 2004). Vaccination will not only reduce the morbidity rate but will also reduce the perinatal mortality and the consequent economic losses caused by the disease.

Vaccination with the reduced dose (1.54×10^5 organisms) of Rev.1 vaccine produced at JOVAC induced a CFT response in all goats. The highest mean CFT antibody titre (1:38) was observed in vaccinated goats two weeks post vaccination, and then declined during the following weeks. This agreed with the work of Alton (1990), who observed that the CFT response began two to three days after vaccination, rising to a peak at two weeks and falling rapidly during the next month. Similarly, our results showed that all goats were seronegative to SAT by 15 weeks post vaccination with a reduced dose of Rev.1 vaccine, which is similar to the work of Alton (1990). The ELISA and the RBPT were the most sensitive serological tests for identifying animals vaccinated with the reduced dose of Rev.1 vaccine throughout the study period.

All serological tests used in this study detected a higher percentage of vaccinated female kids with a full dose than they did in groups vaccinated with a reduced dose or with a conjunctival dose of the vaccine. Similarly, the peak mean CFT and SAT titres were recorded in animals vaccinated with a full dose of Rev.1 vaccine than animals vaccinated with either a reduced dose or with a conjunctival dose of Rev.1 vaccine. This result is in agreement with the findings of Aldomy (1992). Moreover, SAT titres in vaccinated animals with a full dose of Rev.1 vaccine persisted longer than in goats vaccinated with a reduced dose.

The ELISA and the RBPT detected a higher percentage of vaccinated animals than the other serological tests 15 weeks after vaccination in all vaccinated animals with a reduced dose and with a conjunctival dose of the vaccine after 24 weeks, but the CFT, ELISA and the RBPT detected similar percentage (100%) of vaccinated animals with a full dose at two, four, eight and 15 weeks post vaccination. Sera collected from animals and tested by the CFT 15 weeks post vaccination with the full dose and conjunctival dose demonstrated that 100% of animals were seropositive, whereas 0% of animals vaccinated with the reduced dose were seropositive.

The overall results indicated that ELISA (indirect ELISA test) and RBPT were by far the most sensitive serological tests to detect vaccinated animals, either with the full or the reduced dose or the conjunctival (eye drop) dose of Rev.1 vaccine when compared with CFT and SAT. Therefore, the ELISA and RBPT must be considered as screening tests and positive results by these

tests in testing vaccinated animals should be confirmed By CFT.

Peak mean SAT titres were observed 15 weeks post vaccination in goats vaccinated with a full dose, two weeks in goats vaccinated with a reduced dose and four weeks in animals vaccinated with a conjunctival dose. No conclusion could be made from the results of sera collected from vaccinated goats by the use of the SAT. The SAT failed to give a positive result in many goats in which antibody had been detected after natural exposure to vaccine antigen. These observations agree with Zowghi and Ebadi (1985) in Iran, who stated that the CFT proved to be an extremely reliable test for the diagnosis of brucellosis in animals especially where the results of SAT were negative which may occur in the incubation period or in chronic infection.

The overall results suggest that 100% of animals vaccinated with a conjunctival dose of Brucevac vaccine became positive to CFT at two, four, eight and 15 weeks post vaccination, and then the percentage of seropositive animals declined and became 20% at 24 weeks post vaccination. The conjunctival route of vaccination significantly reduced the intensity and duration of the post vaccination serological response and made the use of this vaccine compatible with brucellosis eradication programmes, even when these are based on a test-and-slaughter policy.

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