Immunisation of Adult Sheep Against *Brucella* Infection

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Summary

Experiments were conducted to determine the safe and efficacious vaccinal dose of the live attenuated Strain Rev. 1 of Brucella melitensis, for immunisation of Iranian adult fat-tailed sheep. The experiments revealed that reduction of the normal vaccinal dose to 5 x 10^5 *viable cells confers sufficient immunity without bringing about the undesirable side effects. At this dosage, neither abortion nor excretion of the bacterium via the milk ensued. Besides, serum antibody titres disappeared rapidly enough to allow the routine post vaccination serological tests to be carried out. The efficacy of the vaccine was confirmed by challenge, using either* 3.5 x 10^6 *organisms* of B. melitensis *biovar I Strain 16M or* 3.5 x 10^6 *viable cells of a* B. melitensis *biovar 1 recently isolated from an ovine foetus in Iran. Seven to 8 weeks after infection trials, autopsy and bacteriological examinations of the lymph nodes and tissues verified the results. It was concluded that administration of* 5 x 10^5 *viable germs of Rev. 1 suffice to safely immunise adult sheep against* Brucella melitensis *infection.*

Introduction

Strain Rev.1, a non-dependent reverse mutant selected from a population of streptomycin dependent *Brucella melitensis*, was isolated by Elberg and Faunce (1957). Since isolation, it has been widely used as an efficient live attenuated vaccine strain for prevention of *Brucella* infection in sheep and goats. Many workers have shown that Strain Rev.1 vaccine confers a high degree of protection against experimental and natural challenge with virulent *B. melitensis* (Alton *et al.*, 1967). However, the normal dosage of

1-2 x 10^9 viable cells causes abortion, if used in pregnant ewes and does. Besides, if used during lactation, it may sometimes be excreted in the milk (Alton *et al.*, 1961). Furthermore, when given to adult animals it renders them seropositive for a long time, therefore, making the differentiation between vaccinated animals and those with acquired natural infection difficult. These drawbacks limit the use of Rev.1 vaccine to young virgin lambs and kids only.

Alton (1970) found that when a very small dose, approximately 5 x 10^4 viable cells of Rev.1, was given to pregnant goats the animals did not abort their foetuses, the bacterium could not be detected in the milk and no interference with interpretation of post-immunisation serological tests followed. A significant resistance against *Brucella* infection was conferred upon the inoculated goats. Later, Alton *et al.* (1972) achieved almost identical results when goats were immunised with 3.5 x 10^4 or 7 x 10^8 living cells of Rev.1. Nonetheless, 5 x 10^4 viable cells of Rev. 1 was accepted by FAO/WHO Expert Committee on Brucellosis (1986) as the vaccinal dose for vaccination of adult goats. However, Crowther *et al.* (1977) had not recommended this dosage of vaccine for local adult sheep in Cyprus and, therefore, the vaccinal dose remained uncertain for adult sheep.

In Iran, the efficacy of normal dose of Rev.1 vaccine in protecting sheep against *Brucella* infection had already been proved (Jones *et al.*, 1964; Entessar *et al.* 1967). The present study describes the immunisation of adult fat-tailed Iranian sheep with Rev. 1 vaccine.

Materials and methods

Animals: One hundred Iranian adult fat-tailed ewes of 1.5 to 2 years age and 60 to 65 Kg weight were used. These animals were born and raised in a brucellosis-free environment at the Razi Vaccine and Serum Research Institute (RVSRI) Small Ruminant Breeding Station. At the time of vaccination, all these animals were from 2.5 to 4 months pregnant. Serologically, they had been repeatedly tested and found negative for antibodies to *B. melitensis*. The techniques used in this study were those described and recommended by Alton *et al.* (1975) and Alton *et al.* (1988).

The vaccine: The Strain Rev.1 vaccine was prepared at RVSRI, according to the method described by WHO (1976) and preserved in the freeze-dried status.

Serum samples: Samples were collected before immunisation and, thereafter, once a week during the following 10 weeks. The sampling was continued after challenge for 7 to 8 weeks. All samples were tested by Rose Bengal plate test (RBPT), serum agglutination test (SAT), 2-mercaptoethanol test (2-MET) and complement-fixation test (CFT).

Culture: Aborted foetuses and placentas were examined, by culture, for the presence of strain Rev.1 and for any other known pathogens. The foetuses were autopsied and certain lymph nodes and tissues from organs such as liver, lungs, spleen, as well as the contents of stomachs were removed for culture. From each placenta, four cotyledons were taken for culture. Vaginal swabs and milk samples were taken for culture at the time of lambing or abortion. This was repeated every other day for two weeks and, thereafter, twice weekly for another two weeks. The twice-weekly culture of milk was continued after challenge.

Challenge tests: One month after the last lambing, all the vaccinated sheep plus the controls were challenged with the same number of virulent *B. melitensis* Strain 16M or the Iranian strain biovar 1. The sheep were slaughtered for autopsy seven to eight weeks after challenge. Lymph nodes and selected tissues from each sheep were cultured for *Brucella*. Conventional methods (Alton *et al.*, 1975; Alton *et al.*, 1988; Corbel *et al.*, (1978), for differentiation between Strain Rev. 1 and virulent *B. melitensis* biovar 1, were employed.

Set up of experiments

Experiment 1: The experimental animals consisted of five 10-sheep groups. On 9 July 1994 Group-A,-B,-C, and -D were subcutaneously inoculated with 5×10^4, 1×10^5, 5×10^5 and 1×10^6 viable cells of Rev. 1, respectively. All animals along with those in control group (E) were challenged, on 24 September 1994, by subcutaneous inoculation of $3{,}5 \times 10^6$ of *B. melitensis* Strain 16M. On 13th and 20th November 1994, all animals were slaughtered and autopsied for bacteriological examinations.

Experiment 2: On 4th December 1994, groups F and G, of 20 sheep each, were inoculated subcutaneously with 1×10^5 and 5×10^5 viable cells of Rev. 1, respectively. On 30th April 1995, all these animals along with those in control group H (10 sheep) were challenged by inoculation, subcutaneously, of 3.5×10^6 organisms of an Iranian virulent strain *B. melitensis* biovar 1. On 20th and 29th June 1995, these animals were slaughtered and autopsied for bacteriological examinations .

Results

Serological and bacteriological results, from both experiments, are summarised in Table 1 and Table 2. In **Experiment 1**, all groups were serologically positive, by RBPT and SAT, on Day 14 post-vaccination. The earliest positive serological reaction was recorded on Day 7. The agglutination titres increased for four weeks and then gradually declined. The maximum titres were 1:40, 1:80, 1:320 and 1:320 in Group-A, -B, -C

and -D, respectively. The earliest time that seroconversion, low titres, could be detected by 2-ME and CFT was Day 14 post vaccination. Sera remained positive, by these tests, for up to Week 6 post vaccination. In Group D, the maximum titres of 2-ME and CFT were 1:40 and 1:20, respectively. All serological tests were negative on Week 10, but some became negative as early as Week 7. Only in Group D, 2 abortions occurred 3 to 4 weeks post vaccination. The results of bacteriological examinations of these abortions were negative. All bacteriological tests after natural lambing were also negative. Only in Group D, on one occasion, one sheep excreted 1 colony of Rev.1 in the milk. All vaccinated and control groups showed serological reaction after challenge. The challenge strain could be isolated from 4 sheep in Group A and 1 sheep in Group B. Also, all control non-vaccinated sheep were positive, by culture, for *Brucella*. The percentages of resistance against challenge strain were 70, 90, 100 and 100 in Group-A, -B, -C and -D, respectively. All micro-organisms isolated after challenge were identified as virulent *B. melitensis*.

in **Experiment 2** the serological reactions were almost similar to those in **Experiment 1**. Abortion did not occur in either groups. The results of bacteriological examinations on placentae, vaginal swabs and milk were negative after vaccination. After challenge, two sheep in Group F were shown to be positive, by culture, for *Brucella*. All sheep in Group G were negative in bacteriological tests. The *Brucella* strain was isolated from all sheep in the control group (H). The percentages of resistance against challenge strain were 90 and 100 in Group-F and -G, respectively. Also, all the isolates from the autopsied sheep after challenge were identified as virulent *B. melitensis*.

Discussion

The attenuated B. *melitensis* Strain Rev. 1 vaccine is stable and safe for use in non-pregnant sheep and goats at the age of 3 to 8 months (Alton *et al.*, 1967). However, the normal dose, 1-2 x 10^9 viable cells, may cause abortion or be excreted in the milk, if used in pregnant or lactating sheep and goats (Alton, 1968; Alton, 1970; Jones *et al.*, 1973). In previous experiments in Iran, groups of sheep and goats vaccinated with full dose of Rev. 1 vaccine had during their first pregnancy been challenged along with control group by natural exposure to aborting donors. As shown by autopsies, the vaccinated animals showed significantly higher resistance and, therefore, the vaccine was judged to be effective (Jones *et al.*, 1964; Entessar *et al.*, 1967). Nevertheless, the use of Rev. 1 vaccine in Iran has continued to be limited to young animals, for the reasons mentioned above.

A safe *Brucella* vaccine for adult sheep has always been desired. Alton (1970) reported that a dose of 5 x 10^4 viable cells of Rev. 1 given to pregnant goats did not cause abortion and could not be detected in the milk, although the level of immunity obtained was less than that engendered by the vaccination of young goats with normal dose of the vaccine. In the present study, attempts have been made to evaluate this dose along with three other doses of Rev. 1 vaccine in Iranian fat-tailed sheep. From the only two aborted foetus and their dams, in Group D, neither Strain Rev. 1 nor any other known pathogen could be isolated either at the time of abortion or later. Due to excretion of Strain Rev. 1 into the milk, in one animal of Group D (Table 1), 1 x 10^6 was assumed to be an unsafe dose for adult sheep. Three other groups did not excrete strain Rev. 1 in the milk or at parturition. The serological responses of the sheep in Group-C and -D were very similar and higher than Group-A and -B, although all animals were serologically negative on Week 10 post-vaccination. It is of interest to note that the animals vaccinated as lambs (Alton, 1959) appear to have had higher and longer persisting titres than those vaccinated as adults in our experiment.

The sheep in Group A, that had received 5 x 10^4 viable cells of Rev. 1, did not show satisfactory resistance against challenge with *B. melitensis* Strain 16M; an indication that they had not acquired an acceptable degree of immunity against *Brucella* infection. On the basis of the results obtained in **Experiment 1,** two doses, namely 1 x 10^5 and 5 x 10^5, were chosen for use in **Experiment 2.** All bacteriological tests were negative after vaccination. The serological responses waned in approximately 8 weeks. All sheep in control groups, in either experiments, had a generalised infection as demonstrated at autopsies and proved by culture. On the basis of the degree of resistance, %90 and %100 respectively in Group-F and -G, against challenge strain (Iranian virulent *B. melitensis* biovar 1), 5 x 10^5 viable cells of Rev. 1 was taken to be a safe and efficacious for adult sheep.

However, despite the encouraging results achieved in this study, we are still in agreement with other workers (Crowther *et al.*, 1977; Entessar *et al.*, 1967; Alton *et al.*, 1959; Brinley *et al.*, 1966; Unel *et al.*, 1969) that it is advisable to vaccinate adult sheep 1 to 2 months before mating. Vaccination of adult pregnant ewes in the field should await further experiments.

Table 1. *Experiment 1: Results of bacteriology and serology after vaccination and challenge by* 3.5 x 10 Brucella melitensis, Strain *16M.*

Group	Dose	Weeks. serology positve after vaccin.	No. abortion after vaccin.	No. natural lambing after vaccin.	Bacteri-ology after abortion or lambing	No. excreted Rev.1 in the milk	No. bacteri-ological poitive after lambing	% resisted
A	5×10^4	7	0/10	10/10	Neg.	Nil	3/10	70
B	1×10^5	8	0/10	10/10	Neg.	Nil	1/10	90
C	5×10^5	8	0/10	10/10	Neg.	Nil	0/10	100
D	1×10^6	10	2/10	8/10	Neg.	1/10	0/10	100
E	Control	-	-	10/10	Neg.	-	10/10	0

Table 2. *Experiment 2: Results of bacteriology and serology after vaccination and challenge by* 3.5 x 10 Brucella melitensis, *Iranian srain.*[6]

Group	Dose	Weeks. serology positve after vaccin.	No. abortion after vaccin.	No. natural lambing after vaccin.	Bacteri-ology after abortion or lambing	No. excreted Rev.1 in the milk	No. bacteri-ological poitive after lambing	% resisted
F	1×10^5	8	0/20	20/20	Neg.	Neg.	2/20	90
G	5×10^5	8	0/20	20/20	Neg.	Neg.	0/20	100
H	Control	-	-	10/10	Neg.	Neg.	10/10	0

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