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SEROLOGICAL AND BACTERIOLOGICAL STUDIES OF EWES VACCINATED WITH *BRUCELLA MELITENSIS* STRAIN REV. 1 DURING LACTATION (1)

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RÉSUMÉ

ÉTUDES BACTÉRIOLOGIQUES ET SÉROLOGIQUES CHEZ DES BREBIS VACCINÉES, DURANT LA LACTATION, AVEC *BRUCELLA MELITENSIS* SOUCHE REV. 1

Pendant le premier mois de lactation, 53 brebis ont été vaccinées avec une dose normale de la souche atténuée de *B. melitensis* Rev. 1. La souche vaccinante n'a pu être isolée à partir d'échantillons de lait prélevés chaque semaine pendant 5 semaines après la vaccination. Les titres du Ring-test n'ont été positifs que durant 4 semaines. Dans cette étude, la vaccination par Rev. 1 de brebis en lactation ne produit pas de localisation dans la mamelle.

La réponse sérologique de ces brebis a été comparée à celle de 17 agnelles vaccinées à 8 mois. Les titres présentés par les brebis en lactation étaient légèrement inférieurs à ceux des agnelles durant les 8 premières semaines. Quinze semaines après la vaccination, dans les 2 groupes, le titre moyen déterminé par agglutination était de 51 UI et les réactions de fixation du complément négligeables.

Mots clés : Brucella melitensis, *Rev. 1, Vaccination, Brebis, Lait, Ring-test, Séro-agglutination, Test au rose bengale, Fixation du complément.*

INTRODUCTION

In the course of a study on delayed hypersensitivity reactions in sheep, which will be reported elsewhere (JONES and MARLY, 1975), lactating ewes were given the normal dose of the living attenuated vaccine, *Brucella melitensis* Rev. 1. As little information is available on the excretion of Rev. 1 in the milk of ewes vaccinated during lactation (ALTON and ELBERG, 1967), an attempt was made to isolate the vaccine strain. Milk ring tests were also performed. The serology of the ewes and their suckling lambs was followed for 15 weeks after vaccination.

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MATERIALS AND METHODS

Seventy sheep of the *Préalpes Lacaune* cross in the brucellosis-free flock raised at this Station were vaccinated with Rev. 1 on January 8, 1974. Seventeen ewes were about 8 months of age at the time of vaccination. Fifty-three ewes, 2 to 7 years of age were in lactation. Forty-eight had lambed 3 to 4 weeks before vaccination and 5 had lambed 6 to 9 days before vaccination. Their lambs were kept with them during the experiment.

The Rev. 1 vaccine strain, originally obtained from Dr. S. S. Elberg, University of California, Berkeley, California, was grown on trypticase soy agar. The viable count on the day of inoculation showed that each animal received 7×10^8 viable organisms in the 1 ml dose given subcutaneously.

Foot and mouth disease vaccine was given to all sheep in the flock on March 22, 1974.

Milk samples were taken at weekly intervals for 5 weeks after vaccination. Sampling was discontinued when the lactation period ended. Two ml amounts were removed for the milk ring tests and the remainder was centrifuged. Both the cream and the pellet were cultured on trypticase soy agar with added antibiotics (cycloheximide, bacitracin and polymyxin B in amounts as recommended by ALTON *et al.*, 1975). The ability of the selective medium to initiate the growth of Rev. 1 was determined. Colony counts of the culture on trypticase soy agar with antibiotics was about one half of that on media without antibiotics.

Eight lactating ewes were killed 8 1/2 weeks after vaccination. Portions of 9 lymph nodes, spleen, liver, kidney, uterus and mammary gland were ground and cultured on trypticase soy agar with and without antibiotics.

Sheep were bled 5 days before vaccination, and 6 and 10 days and 2, 4, 8, 11 1/2 and 15 weeks after vaccination. The standard tube agglutination test was performed with 5 p. 100 NaCl as diluent and brucella antigen standardized with International anti-abortus serum. A titer of 1 : 40 was equivalent to 60 International Units. Complement fixation tests were performed on serum samples taken 6 days, 4, 11 1/2 and 15 weeks after vaccination. Sera were inactivated at 60°C for 30 minutes and tests were performed by the micro-titer method described in the U. S. Public Health Monograph (1965) using overnight fixation in the cold. The logarithms of the reciprocal agglutination and complement fixation titers were averaged for each bleeding date for lactating and non-lactating ewes.

The Rose Bengal Buffered Antigen test was performed on some sera with antigen obtained from the Central Veterinary Laboratory, Weybridge, England.

Milk samples from individual sheep were tested undiluted in the milk ring test. Some samples were diluted with pooled milk from non-vaccinated brucella-free sheep. Undiluted and double dilutions beginning with 1 : 2 were prepared in 1 ml volumes. One drop (0.03 ml) of hematoxylin milk ring test antigen was added, tubes were mixed well and incubated at 37°C. Examination was made after 1 and 3 hours. Either a colored cream layer or agglutination at the bottom of the tube was considered a positive reaction.

RESULTS

The results of milk examination by culture and milk ring test are given in table 1. No isolations of brucella organisms were made from the milk of Rev. 1 vaccinated sheep.

Milk ring tests with undiluted milk were positive in 3/4 of samples taken 1 week after vaccination and 1/4 of samples taken 5 weeks after vaccination. Some of the samples taken in the first 3 weeks and all of those taken 4 and 5 weeks after vaccination were diluted in pooled negative milk. Titers as high as 1 : 64 were obtained. The greatest proportion of high titer reactions occurred 2 to 3 weeks after vaccination. At 3 and 4 weeks after vaccination more samples reacted when diluted than when tested undiluted. By 5 weeks after vaccination none of the ewes had a milk ring test titer of 1 : 4.

In tests with milk from brucella infected sheep and goats, agglutination of the stained antigen is usually deposited on the bottom of the tube rather than rising in the cream layer (REPORT, 1971). In the tests with milk from sheep vaccinated with Rev. 1, however, nearly all of the reactions consisted of ring formation within 1 hour of incubation. The second reading, after 3 hours, revealed a few additional reactions. Only 2 samples, taken 4 weeks after vaccination, showed agglutination at the bottom of the tube.

TABLE I

Results of bacteriological and serological examination of milk of ewes vaccinated with B. melitensis *Rev. 1 during lactation*

Résultats des examens bactériologiques et sérologiques du lait des brebis vaccinées avec B. melitensis *Rev. 1 durant la lactation*

Weeks after vacc.	Bacterio-logical results	Undiluted milk test	Total numbers tested	Diluted milk test Number positive at					
				1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
1	0/50 (1)	32/40 (1)	14	3	6	2	0	1	0
2	0/46	32/50	24	1	0	8	6	5	4
3	0/49	20/49	24	1	11	7	2	2	1
4	0/50	22/50	49	18	9	3	2	1	0
5	0/44	10/44	44	5	0	0	0	0	0

(1) Number positive/number examined.

The logarithmic means of the agglutination titers of ewes were determined for each bleeding date and plotted on linear paper (fig. 1). Only those ewes which had not been inoculated with brucella allergen were included. The numbers included at

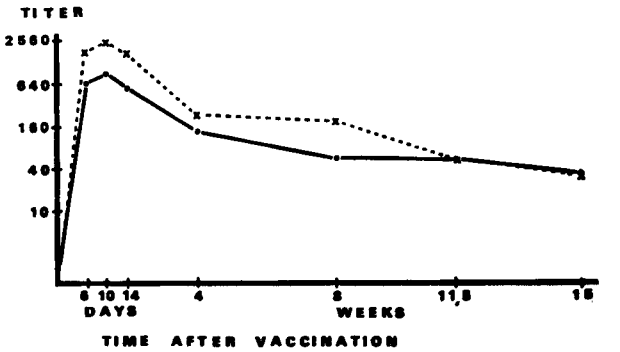

FIG. 1. — *The logarithmic means of tube agglutination test titers of ewes vaccinated with Rev. 1 during lactation* (●——●) *or at 8 months of age* (×-----×)

Moyenne logarithmique des titres déterminés par agglutination en tubes des brebis vaccinées avec Rev. 1 durant la lactation (●——●) *ou à l'âge de 8 mois* (×-----×)

each time period was as follows for non-lactating ewes : 17 for the first 4 weeks, 12 at 8 weeks, 8 at 11 1/2 and 15 weeks. The numbers of lactating ewes was 53 for the first 4 weeks, 38 at 8 weeks and 13 at 11 1/2 and 15 weeks. The figure shows that non-lactating ewes had somewhat higher mean titers than lactating ewes for the first 8 weeks after vaccination. Peak titers observed in the first 2 weeks had dropped at 4 weeks but were still present at a mean titer of 1 : 35 at 15 weeks after vaccination.

The logarithmic means of the complement fixation titers of the two groups of ewes showed a similar trend with non-lactating ewes being somewhat higher. At 15 weeks, however, the mean complement fixation titers were less than 1 : 3 in both groups.

Almost all the sera obtained from ewes in the first four weeks after vaccination were positive in the Rose Bengal test. At 8 weeks after vaccination, 91 p. 100 of the sera from non-lactating ewes and 58 p. 100 of the sera from lactating ewes were positive. At 11 1/2 weeks, 75 p. 100 of the sera from non-lactating ewes and 54 p. 100 of the sera from lactating ewes were positive.

Seventy-nine suckling lambs, bled 2 weeks after their mothers were vaccinated, and 71 lambs, bled 7 weeks after their mothers were vaccinated, had no serological reactions in the tube agglutination test.

The culture of tissues of 8 ewes killed 8 1/2 weeks after vaccination did not reveal the presence of the vaccine strain.

Foot and mouth disease vaccine given 10 1/2 weeks after Rev. 1 vaccination did not appear to interfere with the downward trend of the serological titers.

DISCUSSION

The attenuated vaccine strain *B. melitensis* Rev. 1 gives an effective and lifelong immunity against *B. melitensis* infection in sheep and goats (REPORT, 1971). It is recommended for use in young animals in order to avoid the possibility of causing abortion of pregnant animals or excretion in the milk of lactating animals. Reports of the frequency of excretion of the vaccine strain in the milk vary from none in one study to 1-3 p. 100 in another (ALTON and ELBERG, 1967). In the present study no isolations of Rev. 1 were made from the milk of about 50 ewes examined for 5 weeks after vaccination with Rev. 1. Milk ring dilution tests indicated that vaccination during lactation could interfere with bulk milk ring tests of the flock for four weeks after vaccination but little or no interference would be anticipated later.

Vaccination of ewes during lactation did not cause a more prolonged serological response than that observed in ewes vaccinated at 8 months of age. In both groups of animals the agglutination reactions persisted at a low level but the complement-fixation reactions were negligible at 15 weeks after vaccination. These results confirm that the complement fixation test is the diagnosis test of choice in sheep with a history of vaccination with Rev. 1 (REPORT, 1971).

In sera taken 11 1/2 weeks after vaccination, the Rose Bengal Buffered Antigen test was positive in 62 p. 100 of the sera although the mean tube agglutination titer

at this time was 1 : 52 or 78 IU. This indicates that the Rose Bengal test could only be used as a screening test with confirmation by the complement fixation test in sheep populations where Rev. 1 vaccination is practiced.

The agglutination of stained antigen in milk ring tests with milk from Rev. 1 vaccinated sheep differed from that characteristically seen with milk from infected sheep. This suggests that different immunoglobulins are involved. Little information is available at present on the class of immunoglobulin in sheep milk which causes agglutination of stained brucella antigen.

Although vaccination of lactating ewes with Rev. 1 is not recommended, these studies show that the accidental use of the vaccine in lactating animals is unlikely to cause excretion of the vaccine strain in milk or a serological response more prolonged than observed in other sheep.

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SUMMARY

Fifty-three ewes were vaccinated with the normal dose of the living attenuated strain *B. melitensis* Rev. 1 during their first month of lactation. Attempts to isolate the vaccine strain from milk samples taken weekly for 5 weeks after vaccination were unsuccessful. Milk ring test titers were present for 4 weeks but not for 5 weeks after vaccination. The use of Rev. 1 in lactating ewes did not lead to localization in the mammary gland in this study.

The serological response of these ewes was compared with that of 17 ewes vaccinated at 8 months of age. The titers of lactating ewes were slightly lower than those of non-lactating ewes during the first 8 weeks. At 15 weeks after vaccination the mean agglutination titer was 51 IU for both groups and the complement fixation reactions were negligible.

Key-words : Brucella melitensis *Rev. 1, Vaccination, Ewes, Milk, Ring test, Sero-agglutination, Rose Bengal test, Complement fixation*

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