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THE ISOLATION AND SEROLOGY OF THE "FSA" *BRUCELLA MELITENSIS* REV. 1 MUTANT IN A FLOCK OF SHEEP

S. M. PEFANIS, B. GUMMOW, P. M. PIETERSON, CATHERINE C. WILLIAMSON, CATHARINA G. VENTER and S. HERR, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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A flock of sheep, known to be infected with the "FSA" mutant of *Brucella melitensis* Rev. 1, was examined serologically and bacteriologically to determine whether any relationship existed which would help in the control of this infection in the field. An attempt was also made to determine whether vertical transmission occurred. Twenty-one out of 62 sheep were bacteriologically positive. The best organs for isolation were the udder, supramammary lymphnodes and uterus. No significant relationship could be shown between the complement fixation test and bacterial isolation. The absence of any relationship between serological and bacteriological results agrees with a short-lived infection. None of the 24 lambs sacrificed at 5 months showed either serological reactions or were bacteriologically positive, thus no vertical transmission could be shown.

INTRODUCTION

The Rev. 1 strain of *Brucella melitensis* was first described in 1955 (Herzberg & Elberg), and its biological properties by Elberg & Meyer in 1958 as cited by Alton & Elberg (1967). The use of this vaccine in sheep, was first reported by Renoux in 1958 (Alton & Elberg, 1967), and it was used for the prevention of *Brucella ovis* infection in ewes and rams in South Africa for the first time in 1960 (Van Drimmelen). The Rev. 1 vaccine is still currently in use in the Republic of South Africa to control *B. ovis* infection in rams. The immunity resulting from Rev. 1 vaccination is long-lasting and persistence of Rev. 1. in sheep is less than 6 months although this varies with the age, pregnancy status at vaccination, and the breed (Alton, 1985a). According to Alton (1985a), following the vaccination of virgin kids and lambs, it has never been possible to recover Rev. 1 from the udder or the vagina after parturition. The vaccination of pregnant sheep or goats is likely to cause abortion with the excretion of Rev. 1, although this excretion does not last as long as that which follows an abortion with a field strain of *B. melitensis* (Alton, 1985a). Neeman, cited by Alton (1985a) was unable to produce changes in the virulence of Rev. 1 by passage through pregnant sheep. He also demonstrated that sheep excreting Rev. 1 at abortion were unable to transmit infections to susceptible pregnant sheep in close contact. According to Bosseray (1985) it appears as if strains of Rev. 1 in use in different parts of the world, differ considerably in immunogenicity but no lateral or vertical spread of Rev. 1 has been reported.

The first isolate of the "FSA" mutant form of *B. melitensis* Rev. 1 was received at this laboratory from Natal during March 1984. Subsequent to this, isolates from sheep, goats, 2 humans and 2 batches of "Rev. 1" vaccine were received from the Cape Province and Transvaal in 1985 and 1986. The characteristics of this variant strain of Rev. 1 are described by Pieterson, Gummow, Pefanis, Venter & Herr (1988). The "FSA" strain of *B. melitensis* resembles Rev. 1 in its reactions to penicillin and streptomycin but reacts closer to a field strain of *B. melitensis* as regards dye sensitivity and colony size (Pieterson *et al.*, 1988). The current investigation deals with the serological and bacteriological findings in a flock of sheep in which abortions had occurred and this variant strain of Rev. 1 had been isolated from the abortion material. The flock of sheep was acquired with a view to attempting to correlate the serological results with isolation successes in order to simplify control of this infection in the field. Whether vertical transmission to the progeny occurred was a further question which required elucidation.

MATERIALS AND METHODS

Animals

The flock of sheep, with no history of any *Brucella* vaccination, consisted of 60 ewes, 2 rams and 24 suckling lams. Ten of the 60 ewes were still pregnant. Eleven of the ewes had already aborted at the time the flock was purchased. Serum was collected twice, with a 5 day interval, from all the sheep just prior to slaughter. The adults were euthanased with intravenous sodium pentobarbitone[1] and a *post mortem* examination was performed on each animal. Samples of lung, liver, spleen, left and right udder, left and right supramammary lymphnodes, iliac lymphnodes, internal inguinal lymphnodes, ovaries or testes, uterus or epididymis, brain, heart and muscle from the pillars of the diaphragm were collected aseptically. Organs were also collected from the foetuses removed from the pregnant ewes. Samples collected from foetuses were: lung, liver, spleen, kidney, stomach contents, abomasum, rumen, brain and placenta. Serum was also collected from the 24 lambs at 5 months of age. These lambs were then slaughtered and the same set of organs as for the adult sheep were collected.

Bacterial isolation and culture media

A bacteriological loop was used to plate out material taken from the freshly-cut surface of each organ. Five different media were used throughout the study, as described by Herr & Roux (1981), and each specimen was plated out on all 5 media. Plates were incubated at 37 °C in air and air plus 10 % CO_2. Plates were examined macroscopically and microscopically for the presence of typical *Brucella* colonies after 48, 96 and 114 h.

Typing of isolates

All the isolates were typed as described by Corbel & Hendry (1983), except for a few modifications as described by Pieterson *et al.* (1988).

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[1] Eutha-naze, Centaur Labs (Pty) Ltd, 36 Durban St., Johannesburg 2001

TABLE 1 Organs from which *B. melitensis* Rev. 1 FSA were isolated

Animal No.	Lung	Liver	Spleen	Udder l.	Udder r.	Supramammary l.	Supramammary r.	Iliac	Inguinal l + r	Ovaries	Uterus	Brain	Heart	Muscle	Foetus
1				+	+	+	+			+	+				
6		+	+	+					+		+		+	+	
7															+
12	+			+				+			+	+		+	
14			+		+		+			+	+				
21			+	+	+	+	+	+	+	+	+				
22															+
23							+				+				
26	+						+								
27							+	+							
28				+	+	+	+				+	+			
34							+								
41										+					
49		+													
51			+	+	+	+	+				+				
53		+	+		+	+							+		
54								+							
55		+									+		+		
57		+													
58					+	+								+	
59	+														
Total	3	5	5	6	7	6	9	4	2	4	9	2	3	3	2

Serology

The complement fixation test (CFT) technique was as used for bovine brucellosis at the Veterinary Research Institute, Onderstepoort. The method was similar to that used by the Central Veterinary Laboratory, Weybridge (Morgan, Mackinnon, Gill, Gower & Norris, 1978), but adapted to microtitration by Herr, Bishop, Bolton & Van der Merwe (1979). The results are reported in International Units per millilitre (IU/mℓ) (Herr, Williamson, Prigge & Van Wyk, 1986).

RESULTS

All the isolates were typed as the mutant "FSA" strain of *B. melitensis* Rev. 1 as described by Pieterson *et al.* (1988). Nineteen of the 62 sheep were positive bacteriologically for *Brucella*. A further 2 sheep were classified as positive for *Brucella* after the organism was isolated from the foetuses of these 2 ewes. Only 2 out of the 10 foetuses examined were positive for "FSA" on culture. None of the 24 lambs at 5 months showed any serological reaction or proved positive on isolation.

Table 1 shows from which organs *Brucella* was isolated in each of the 21 sheep.

Table 2 shows the relationship between the CFT and the isolation of *Brucella* from the adult sheep. A titre of less than 60 IU/mℓ was designated a low titre, 60–196 IU/mℓ a medium titre and more than 196 IU/mℓ a high titre.

Ten of the sheep from which *Brucella* was isolated had titres of less than 60 IU/mℓ in the CFT test.

The dams of the 2 positive foetuses were bacteriologically negative, although the dams showed CFT titres of 480 and 784 IU/mℓ respectively. Both the rams had CFT titres of less than 60 IU/mℓ and both were negative on bacterial isolation.

TABLE 2 Relationship between CFT titre and results of bacterial isolation

Serology CFT titre (IU/mℓ)	Bacterial isolation	
	Positive	Negative
196	10	13
60–196	1	5
60	10	23
Total	21	41

TABLE 3 The relationship between rising, falling and consistent CFT titres and bacterial isolation

Serology CFT titres	Bacterial isolation	
	Positive	Negative
2–4 × rising titre	8	8
2–4 × falling titre	2	13
Consistent titre	11	20
Total	21	41

Table 3 shows the relationship between a changing CFT titre and bacterial isolation. A rising titre or consistent titre should theoretically indicate an active ongoing infection whereas a falling titre could possibly indicate prior exposure.

No further isolations of FSA have been reported from the field to date (January 1988).

Discussion

Although no history of inoculation with any *Brucella* vaccine was reported the authors cannot guarantee that vaccination did not occur. However, if *Brucella* vaccination had not occurred then the "FSA" mutant strain is capable of being laterally transmitted.

The fact that none of the lambs, slaughtered at 5–6 months old, showed any serological reaction or were positive on bacteriological tests, would indicate that there was no vertical transmission of this mutant form of *B. melitensis* Rev. 1 (FSA).

The most common organs from which isolates were made were the udder, supramammary lymphnodes and uterus which is in accordance with other authors (Alton, 1985b; Herr & Roux, 1981). This could be of zoonotic importance in the abattoir industry as the strain has already shown itself to be pathogenic in man (Pieterson *et al.*, 1988). The isolation of *B. melitensis* from the ovaries in 4 out of 19 of the sheep was noteworthy as was the isolation from the heart muscle in 3 out of 19 cases, because these organs are not generally referred to in the literature as suitable for isolation of *Brucella*. The isolation of *Brucella* from the lung tissue only in animal number 59 may be indicative of a very early infection and point to inhalation infection.

Serological testing does not appear to be a very reliable method of indicating "FSA" infected animals in the flock. The specificity of the CFT test calculated according to Cutler (1979) was 56 % and the sensitivity was 52 %. The predictive value of the CFT was calculated at 58 %. The aim of Table 3 was to examine the possibility that a rising or consistent titre would indicate active ongoing infection whereas a falling titre could indicate prior exposure to *Brucella* with subsequent elimination of the infection. The difference between twofold rise and twofold decrease in titre as an indicator of infection is also of no significance as the results were equivocal. The absence of any significant relationship between serological and bacteriological results is difficult to explain except in terms of a short-lived bacterial infection which is reported for Rev. 1 (Alton, 1985a). This also agrees with the apparent failure of the "FSA" strain to maintain infection in the field, as witnessed by the absence of further recoveries of "FSA" to date (January 1988).

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