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RESEARCH COMMUNICATION

THE ISOLATION AND SEROLOGY OF *BRUCELLA MELITENSIS* IN A FLOCK OF GOATS IN CENTRAL RSA

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ABSTRACT

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Brucella melitensis biotype 1 was isolated in pure culture from the lungs, liver, spleen, kidney, stomach contents, abomasum and brain of an aborted caprine (Boer goat) foetus in the district of Cullinan near Pretoria. The 18 does and 1 ram in the flock of Boer goates were examined serologically by means of the complement fixation (CF) test, using *Brucella abortus* antigen. Six weeks later they were examined again, using *B. abortus* as well as *B. melitensis* biotype 1 antigens. No significant differences were found between the 2 CF tests using *B. abortus* antigen, or between the results obtained by using the *B. abortus* and *B. melitensis* antigens.

Twelve goats, showing CF antibody titres, were slaughtered and examined bacteriologically. No relationship was found between the serological and bacteriological results.

INTRODUCTION

Brucella melitensis is an infectious disease affecting mainly sexually mature sheep and goats, but the organism is highly pathogenic and readily affects cattle and other animals. It is also an important zoonosis, and man is often infected following contact with infected animals or the consumption of contaminated milk and milk products (Alton, 1985). Various breeds of goats differ little in their susceptibility to *B. melitensis* infection, contrary to what is the case in sheep (Alton, 1985; 1987; Joint FAO/WHO, 1986). The principal symptom is abortion during the later stages of pregnancy. The male plays an insignificant role in the transmission of the disease. Infection usually takes place via the nasopharynx, by inhalation of infectious aerosols, or by ingestion, though penetration of the skin can occur, especially if it is abraded. The main source of infection is the vast numbers of bacteria present in genital discharges following an infected birth or abortion (Alton, 1985).

The first proven *B. melitensis* of animal origin in southern Africa was reported by Van Drimmelen in 1953, when the organism was isolated from Karakul sheep in South West Africa (Van Drimmelen, 1953). In 1965, the same author reported the isolation of *B. melitensis* biotype 1 from 3 outbreaks of abortion in sheep flocks, 2 in the Eastern Transvaal and 1 in the Northern Transvaal (Van Drimmelen, 1965). In contrast with these isolations, the infection has not been found in the Republic of South Africa for over 20 years. There were, however, 3 isolates made from humans in the northern parts of SWA/Namibia and identified as *B. melitensis* biotype 1[1] (Herr, unpublished data, 1988). The same biotype was recently isolated from a goat in the same area[2] (Ribeiro, unpublished data, 1989). The only other isolations of *B. melitensis* in the RSA were of the Rev. 1 vaccine strain and of the "FSA" mutant of the vaccine strain (Pieterson, Gummow, Pefanis, Venter & Herr, 1988; Hunter, Pefanis, Williamson, Botha & Van Schalkwyk, 1989).

In this report are discussed the serological and bacteriological findings in a flock of Boer goats in which abortions took place. *B. melitensis* biotype 1 was isolated from an aborted foetus, a pregnant doe and her unborn foetus.

MATERIALS AND METHODS

Specimens

The flock of goats consisted of 18 does and 1 ram with no history of vaccination, which had been purchased by the present owner at a public auction 1 year previously. Five of the 7 pregnant does aborted, the foetus of the last aborting doe being examined bacteriologically. Foetal samples used for this investigation consisted of lung, liver, spleen, kidney, stomach contents, abomasum and brain. The samples were kept on ice and cultured within 1 h of collection. Serum, collected twice, with a 6-week interval, from all 19 animals, was examined serologically. Twelve does which had CF antibody titres were slaughtered. During a post-mortem examination, samples of left and right udder, left and right supramammary lymphnodes and middle iliac lymphnodes were collected aseptically for bacteriological examination. Samples of placenta and foetal stomach contents were also collected from the 2 pregnant does which had not aborted.

Serology

The complement fixation (CF) test technique, using either *B. abortus* (strain 99) or *B. melitensis* biotype 1 (Rev. 1) antigens, was performed as for bovine brucellosis at the Veterinary Research Institute, Onderstepoort. This method is similar to that used by the Central Veterinary Laboratory, Weybridge (Morgan, Mackinnon, Gill, Gower & Norris, 1978), but adapted to microtitration (Herr, Bishop, Bolton & Van der Merwe, 1979). The results were expressed in international units per millilitre (IU/mℓ) (Herr, Williamson, Prigge & Van Wyk, 1986).

Bacterial isolation and culture media

Material taken from the freshly-cut surface of each organ with a bacteriological loop was plated out onto each of 5 different media, namely tryptose agar plus 10 % horse blood, tryptose agar plus 10 % horse blood and antibiotics, brain/heart infusion agar plus 10 % horse blood, *Brucella* agar plus 5 % horse blood, and serum dextrose agar plus antibiotics, as described by Herr & Roux (1981). Culture plates were incubated at 37 °C in air and air plus 10 % CO_2, and examined for the presence of *Brucella* colonies after 48, 96 and 144 h.

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[1] Isolates submitted for typing by Dr A. C. Mauff, P.O. Box 8475, Johannesburg 2000

[2] Culture submitted for typing by the Central Veterinary Laboratory, Windhoek, SWA/Namibia

Typing of isolates

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

RESULTS

Bacteriological examination of the aborted foetus yielded *Brucella* organisms from all the organs and in all the different media used in this investigation. These isolates were typed as *Brucella melitensis* biotype 1.

When first tested, 12 of the 18 does had CF antibody titres ranging between 21 and 392 IU/mℓ, using *B. abortus* antigen. Six weeks later, 11 of the does had CF titres ranging between 30 and 392 IU/mℓ when *B. abortus* antigen was used, and between 18 and 392 IU/mℓ using *B. melitensis* antigen (Table 1). The other doe, Doe 12, previously showing a CF titre of 21 IU/mℓ, had no titres in the last 2 tests. The remaining 6 does had no titres in any of the 3 tests. Ram 13 showed no CF titre in the first test, but had titres of 60 and 98 IU/mℓ, respectively, in the 2nd test, using *B. abortus* antigen and in the test with *B. melitensis* antigen. In the space of 6 weeks, the ram and 5 does showed rising CF titres, 4 does had falling titres, and the remaining 3 does had consistent titres. When comparing the results obtained by using *B. abortus* antigen with those obtained with *B. melitensis* antigen, 7 does showed the same CF titres on both tests, 4 had lower titres with *B. melitensis* antigen, and 1 doe and the ram had higher titres with *B. melitensis* antigen.

TABLE 1 Serological titres with *B. abortus* antigen tested 6 weeks apart and with *B. melitensis* antigen

Animal Nos	Serological CF titres (IU/mℓ)		
	B. abortus (1st)	*B. abortus* (2nd)	*B. melitensis*
1	392	196	196
2	240	240	392
3	196	392	392
4	196	240	196
5	196	196	98
6	172	98	98
7	49	98	98
8	49	49	18
9	49	30	30
10	21	43	43
11	21	30	24
12	21	–	–
13	–	60	98
14–19	–	–	–

On bacteriological examination of the organs collected from the 12 slaughtered does, *Brucella melitensis* biotype 1 was isolated from the middle iliac lymphnodes and foetal stomach contents from 1 pregnant doe. No further isolations were made.

DISCUSSION

Although no history of inoculation with any *Brucella* vaccine was reported, there is no guarantee that vaccination did not take place. Nevertheless, no *B. melitensis* Rev. 1 vaccine strain organisms were isolated. The typing of the isolates from the foetus and 1 of the slaughtered does as *B. melitensis* biotype 1 strongly indicates that this strain was the cause of the abortions and the infection, as indicated by the CF tests results.

The isolation of *B. melitensis* from only 1 of the slaughtered does, in spite of the fact that all 12 showed CF antibody titres and 5 had aborted, was unexpected. This result may have been affected by the rather limited range of organs collected for bacteriological examination. Nevertheless, the choice of organs was based on previous reports that those were amongst the most common organs from which isolations were made (Herr & Roux, 1981; Alton, 1985; Pefanis, Gummow, Pieterson, Williamson, Venter & Herr, 1988). It is arguable that further isolations could have resulted from the examination of a wider range of organs (Corner, Alton & Iyer, 1987).

The serological results obtained with *B. abortus* antigen show only a small variation in the CF titres over a period of 6 weeks. This may indicate that CF titres in goats do not rise as high as those experienced in cattle. The closeness of the CF test results obtained with *B. abortus* and *B. melitensis* antigens indicates that both antigens are equally suited to detect antibodies against *B. melitensis* infection, at least when using the S99 and Rev. 1 strains.

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