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RESEARCH COMMUNICATION

A SEROLOGICAL COMPARISON OF COMPLEMENT FIXATION REACTIONS USING *BRUCELLA ABORTUS* AND *B. MELITENSIS* ANTIGENS IN *B. ABORTUS* INFECTED CATTLE

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ABSTRACT

HERR, S., LAWRENCE, JANET V., BRETT, O. L. & RIBEIRO, L. M. M., 1991. A serological comparison of complement fixation reactions using *Brucella abortus* and *B. melitensis* antigens in *B. abortus* infected cattle. *Onderstepoort Journal of Veterinary Research*, 58, 111–114 (1991).

Brucella abortus and *B. melitensis* antigens were used in parallel on the National Standard *Brucella abortus* antiserum and on field sera coming from cattle where practically exclusively *B. abortus* biotypes 1 and 2 have been isolated over the last 11 years. With the National Standard serum the titres to *B. melitensis* were consistently lower than those to *B. abortus* antigen. Most were 1 dilution (twofold) lower. Although a similar trend was seen with the field sera, there were 7/346 sera which had twofold or higher titres to *B. melitensis* antigen. Although this may be due to the vagaries of the test it also warrants closer investigation of the animals concerned to see whether M-antigen predominant *Brucella* biotypes are possibly present. The use of the dual antigens could identify herds which are infected only with A-antigen predominant brucellae but would not be reliable for classifying individual animals.

INTRODUCTION

An isolated outbreak of *Brucella melitensis* infection in 2 goats on the same property after the disease had been absent from the Republic of South Africa (RSA) for more than 20 years (Ribeiro, Herr, Chaparro & Van der Vyver, 1990) is a matter for concern. The danger of spread to the cattle population where there is mixing with infected sheep and goats is amply documented (Verger, 1985). Should *B. melitensis* infection occur in cattle, the capability to recognize the condition and differentiate it serologically from *B. abortus* infection would be advantageous.

Serological testing for the presence of *B. melitensis* infection is often reliant on the use of the standard *B. abortus* antigens (Waghela, 1978; Kolar, 1984; Verger, 1985; Alton, Jones, Angus & Verger, 1988). The use of *B. melitensis* antigens to detect *B. melitensis* biovar 1 or other M-antigen rich *Brucella* strains has been suggested and marginally successfully demonstrated in agglutination and complement fixation (CFT) tests (Strauch, 1960; Corbel, 1985), while a marginal difference in *B. abortus* infected animals was also described using *B. abortus* and *B. melitensis* antigens (Alton, 1971).

The CFT is used throughout the RSA as the definitive test in bovine, ovine and caprine brucellosis. No clear-cut serological differentiation could be detected, using the 2 antigens in the CFT, in *B. melitensis* infected goats (Ribeiro *et al.*, 1990). In preparation for the possible spread of *B. melitensis* infection to cattle in the future, it was decided to investigate the serological difference, if any, in the CFT between the 2 antigens in a known *B. abortus* infected cow serum and in bovine sera from the field where only *B. abortus* infection in cattle has been observed for the last 20 years. This would serve as preparatory information with which serological results from future *B. melitensis* infection in cattle could be compared.

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MATERIALS AND METHODS

The National Standard *Brucella abortus* antiserum[1] with a titre of 1 400 International Units per mℓ (IU/mℓ) in the CFT, was diluted 1/5,6 to give a serum with 250 IU/mℓ. This serum has the same agglutination titre with the standard and 2-mercaptoethanol *Brucella* serum agglutination tests. This indicates that mainly IgG is present in this serum, which is a criterion recommended for Standard sera (Joint FAO/WHO Expert Committee on Brucellosis, 1986). The serum came from a naturally infected (*B. abortus* biotype 1) cow (Herr, Ribeiro & Chaparro, 1990). The National Standard serum was tested 81 times on various occasions in the CFT done as described by Herr, Bishop, Bolton & Van der Merwe (1979) but using *B. abortus*[2] *and B. melitensis* antigens. The *B. melitensis* antigen was prepared as described by Ribeiro *et al.* (1990). The Rev. 1 strain was used as suggested by Alton *et al.* (1988). Production parameters were the same as for the commercial *B. abortus* antigen and contained a packed cell volume of 5,3 %. This was diluted with veronal buffer (Herr *et al.*, 1979) and standardized by chequerboard titration against the 250 IU/mℓ dilution National Standard antiserum. The optimum dilution proved to be 1/100 and this dilution of antigen was used throughout. Titres for both antigens were recorded in IU/mℓ from a table where 50 % haemolysis in a 1/220 dilution is equivalent to 1 000 IU/mℓ (Herr, Williamson, Prigge & Van Wyk, 1986). Twofold serum dilutions between 1/4 and 1/128 were used in the test, the 1/2 dilution was used as anticomplementary control and the highest titre would be 784 IU/mℓ.

Bovine sera which were submitted for routine brucellosis testing were subjected to the Rose Bengal test (RBT) done in haemagglutination trays

[1] *B. abortus* standardized antiserum, Veterinary Research Institute, Onderstepoort

[2] *B. abortus* complement fixation antigen, Veterinary Research Institute, Onderstepoort

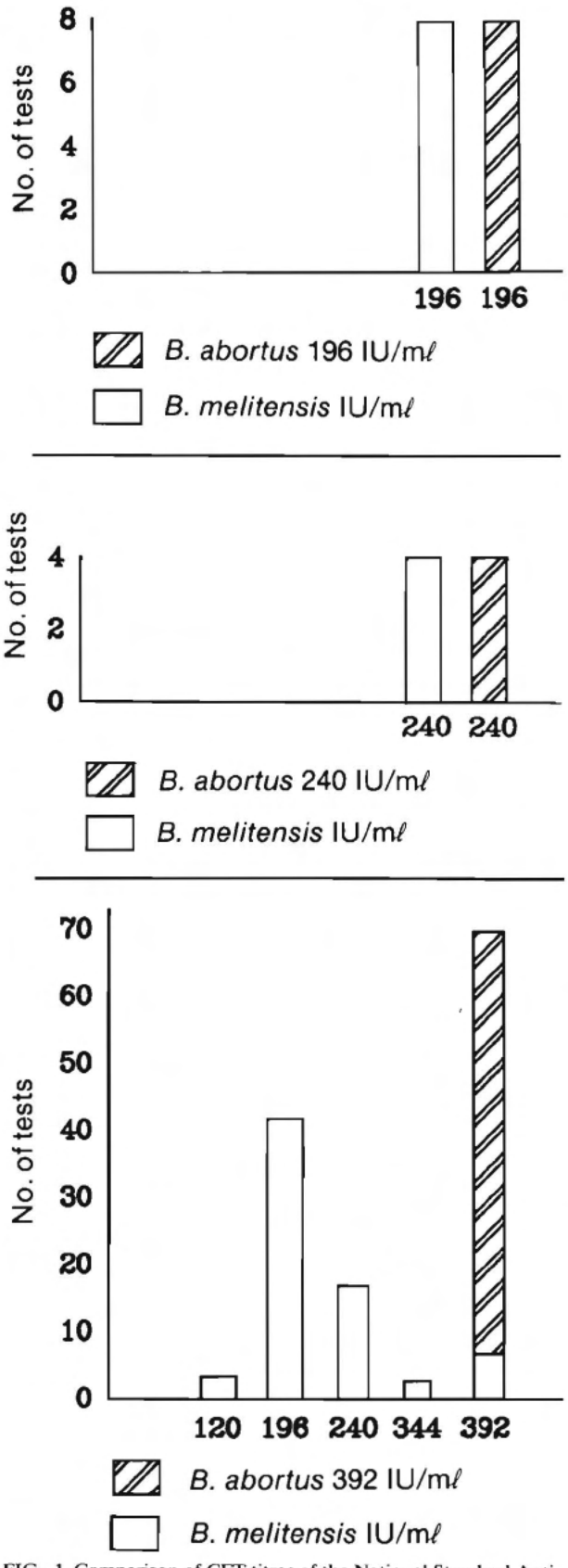

FIG. 1 Comparison of CFT titres of the National Standard Antiserum recorded with *B. abortus* and *B. melitensis* antigens

(Anon., 1980) using 25 µℓ serum and 25 µℓ atigen[3]. Four hundred and ninety-six RBT-positive sera were subjected to the CFT using the 2 different antigens, as above. Only sera with endpoint titres of 392, 240, 196, 98, 49, 24 or ⩽ 15 IU/mℓ in the *B. abortus* test were compared with whatever their titres happened to be in the *B. melitensis* test. The sera with titres of 784 IU/mℓ were ignored as this did not reflect their true endpoint. Too few sera with other titres were found to be of use in comparisons.

RESULTS

The titres found with the *B. melitensis* antigen at different levels of *B. abortus* antigen endpoints are recorded for the National Standard antiserum in Fig. 1 and for the field sera in Fig. 2.

DISCUSSION

The decision to use the Rev. 1 strain of *B. melitensis* as recommended by Alton *et al.* (1988) rather than the more virulent strains used by Strauch (1960) and Corbel (1985) was taken because of its lower pathogenicity with a view to commercial production.

The general trend of finding the sera reacting to *B. melitensis* antigen at approximately 1 dilution (twofold) lower than with the *B. abortus* antigen (Fig. 1 and 2) is in agreement with other comparative serological results (Strauch, 1960; Corbel, 1985) but contrary to the findings of Ribeiro *et al.* (1990) where no serological distinction could be made in goat sera with the presence of *B. melitensis* infection, using the same 2 antigens.

The reproducibility of the test results were well within the two- to fourfold range as reported by Herr, Roux & Pieterson (1982) as tests on the National Standard antiserum show (Fig. 1).

Only *B. abortus* has been isolated from cattle in the RSA over the last 20 years and during the last 11 years approximately 90 % of isolations were biotype 1, 10 % biotype 2 and a single isolate biotype 3 (unpublished laboratory data 1980–90). All these biotypes are A (abortus) antigen predominant as opposed to M (melitenses) antigen predominant brucellae such as *B. abortus* biotypes 4, 5 and 9 and *B. melitenses* biotype 1 (Alton *et al.*, 1988). It was therefore surprising to find even the small number (7) of field sera that showed twofold or higher titres to the *B. melitensis* antigen (Fig. 2). Although this may merely be due to the vagaries of the test (Herr *et al.*, 1982), such animals deserve further investigation to determine which *Brucella* species and biotypes may be present.

The general pattern observed with *B. melitensis* antigen showing lower, and mostly twofold lower titres could be used on a herd basis to demonstrate the involvement of A-antigen predominant brucellae. Individual animals cannot, from these results, be classified serologically as being infected with either the A- or M-antigen predominant biotypes before further work clarifies the anomalies of the higher *B. melitensis* titres.

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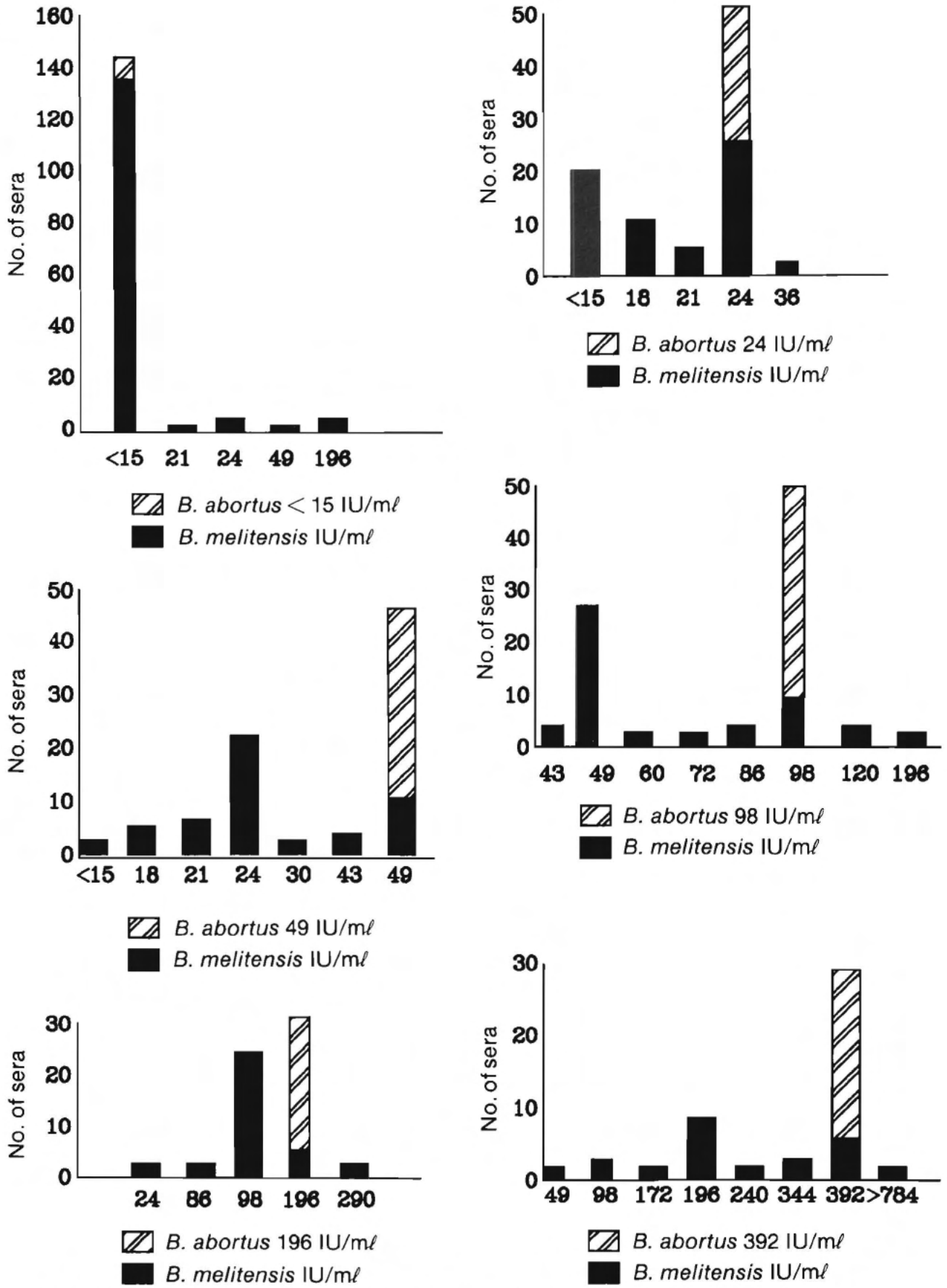

FIG. 2 Comparison of CFT titres in field sera recorded with *B. abortus* and *B. melitensis* antigens

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