RESEARCH COMMUNICATION

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

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ABSTRACT


Brucella abortus and B. melitensis antigens were used in parallel on the National Standard’s Brucella abortus antigen 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 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.

Sero logical 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 the 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.

MATERIALS AND METHODS

The National Standard Brucella abortus antisera 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 5.2 %. This was diluted with veronal buffer (Herr et al., 1979) and standardized by chequerboard titration against the 250 IU/ml dilution National Standard antisera. 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 and a titre of 1/220 dilution is equivalent to 1 000 IU/ml (Herr, Williamson, Fridge & Van Wyk, 1966). Twofold serum dilutions between 1/4 and 1/28 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.
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 antisera 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 & Pieterse (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 (melitensis) antigen predominant brucellae such as B. abortus biotypes 4, 5 and 9 and B. melitensis 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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REFERENCES


1 B. abortus Rose Bengal antigen, Veterinary Research Institute, Onderstepoort


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