

AN ELISA USING AN SDS EXTRACT OF *BRUCELLA ABORTUS* STRAIN 99 AS ANTIGEN TO DETECT *B. ABORTUS* ANTIBODIES IN CATTLE SERA

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ABSTRACT

WILLIAMSON, CATHERINE C., OBEREM, P. T., POERSTAMPER, C., DE WAAL, D. T., MATTHEE, O. & BRETT, O. L., 1988. An ELISA using an SDS extract of *Brucella abortus* strain 99 as antigen to detect *B. abortus* antibodies in cattle sera. *Onderstepoort Journal of Veterinary Research*, 55, 1-3 (1988).

An ELISA was developed using an SDS extract of *Brucella abortus* as antigen to detect antibodies in cattle sera. The antigen was stable at 4 °C for at least 4 years and although it gave optimal results at a 1/4 000 dilution it could detect reactors at a 1/32 000 dilution. Based on comparative CF tests on 430 sera from negative herds and 187 sera from positive herds a reading of 0,07 or less using a 492 nm filter could be considered a negative reaction.

The ELISA could be a useful test to supplement the CF test but further evaluation is still required.

INTRODUCTION

The complement fixation test (CFT), though a reliable test for bovine brucellosis cannot be used on anticomplementary or badly haemolyzed sera. Complement fixing activity is associated with immunoglobulin G 1 fraction III and immunoglobulin M, but no complement fixing activity is associated with immunoglobulin G 2 (Patterson, Deyoe & Stone, 1976). For these reasons, a supplementary test that circumvents the problem of anticomplementary sera and detects immunoglobulins other than the CFT was investigated. An enzyme-linked immunosorbent assay (ELISA) has further advantages in that only minute amounts of antigen are required and it is readily adaptable to large scale screening. ELISA systems for bovine brucellosis have been described by Lamb, Jones, Shurig & Berman (1979), Byrd, Heck & Hidalgo (1979) and Sutherland (1985), but an ELISA using a sodium dodecyl sulphate (SDS) extract of *Brucella abortus* strain 99 has not hitherto been described.

MATERIALS AND METHODS

Sera

Four hundred and thirty sera from 3 herds known to be negative on repeated CFT tests for brucellosis were used. In 2 of these herds calfhood vaccination with Strain 19 had been applied and the third was unvaccinated and free of brucellosis. One hundred and eighty-seven sera originated from herds classed as positive for brucellosis using CFT reactions as criteria. CFT reactions of 30 IU/ml or higher were considered positive in these herds (Herr, Roux & Pieterse, 1982).

Complement fixation test (CFT)

The CFT was carried out in microtitre plates as described by Herr, Huchzermeyer, Te Brugge, Williamson, Roos & Schiele (1985).

Enzyme-linked immunosorbent assay (ELISA)

Reagents

Positive control serum was obtained from a cow from which *Brucella abortus* biotype 1 had been isolated. The serum was diluted with negative serum to give a titre of 196 IU/ml on CFT, freeze-dried in 1 ml aliquots and stored at 4 °C.

Negative control serum was obtained from an animal from a negative herd. It was likewise freeze-dried and stored.

Antigen was an SDS extract of *Brucella abortus* Strain 99 prepared by using a modification of the method described by Raybould & Chantler (1979).

Brucella abortus Strain 99 was seeded onto tryptose agar containing 5 % equine serum and 1 % glucose. Single colonies were subcultured at 5-7 days. After the 3rd subculture, the growth on 3 Petri dishes was suspended in phosphate buffered saline (pH 6,4) and inoculated into 1 l of "Marmite" medium which was made up by adding 100 g of Marmite⁽¹⁾, 200 g of peptone, 300 g of glucose, 16,25 g of Na₂HPO₄·12H₂O to 10 000 ml of distilled water. This was stirred on a magnetic stirrer for 18 h at 37 °C and then inoculated into a fermenter with 9 l of "Marmite" medium, stirred at 300 r.p.m. and aerated at 2 l/min, for 24 h, then 4 l/min for a further 48 h, after which the culture was sedimented by means of carboxymethyl cellulose⁽²⁾. The culture was allowed to sediment overnight at 4 °C, the sediment was inactivated for 25 min at 60 °C in a water-bath and then for 60 min at 60 °C in a hot-air oven. Phenol was added to 0,5 % m/v and NaCl to a final concentration of 0,85 %. The material was filtered through a Zeta plus grade 01A filter⁽³⁾.

The suspension was centrifuged for 20 min at 21 500 g. The supernatant was discarded and the sediment resuspended to 20 % v/v in 0,01 M phosphate buffered saline (PBS), pH 7,5. One-twentieth of volume of 10 % m/v SDS was added to 1 volume of 20 % v/v bacterial suspension in PBS. This was stirred gently for 24 h at 4 °C and then centrifuged for 20 min at 21 500 g to sediment the bacterial cells. The supernatant was removed and filtered through depth type filters⁽⁴⁾ of increasing density until clear. The antigen was stored at 4 °C and was titrated by chequer-board titration for the ELISA test.

Buffers. 0,1 M carbonate bicarbonate buffer (pH 9,6) was used as coating buffer and 0,01 M phosphate buffered saline (pH 7,6) as incubation buffer, and 0,1 M citrate phosphate buffer (pH 5) was used to dissolve the substrate. The washing solution consisted of 0,85 % saline with 0,5 % Tween 20.

Conjugate: Peroxidase conjugated rabbit immunoglobulins to cow immunoglobulins⁽⁵⁾ diluted 1/1 500 with incubation buffer according to titration was used.

⁽¹⁾ Marmite (SA) (Pty) Ltd, 5 Parsons Street, Industria, Johannesburg

⁽²⁾ Hercules Powder Company, Wilmington 99, Delaware, USA

⁽³⁾ Filterklean Liquid Conditioning Co. 4 Lawn St, Rosettenville 2190

⁽⁴⁾ Seitz Industries South Africa, P.O. Box 11, Parowvallei 7503

⁽⁵⁾ Dakopatts, 42 Produktionsweg, P.O. Box 1359, DK-2600 Glostrup, Denmark

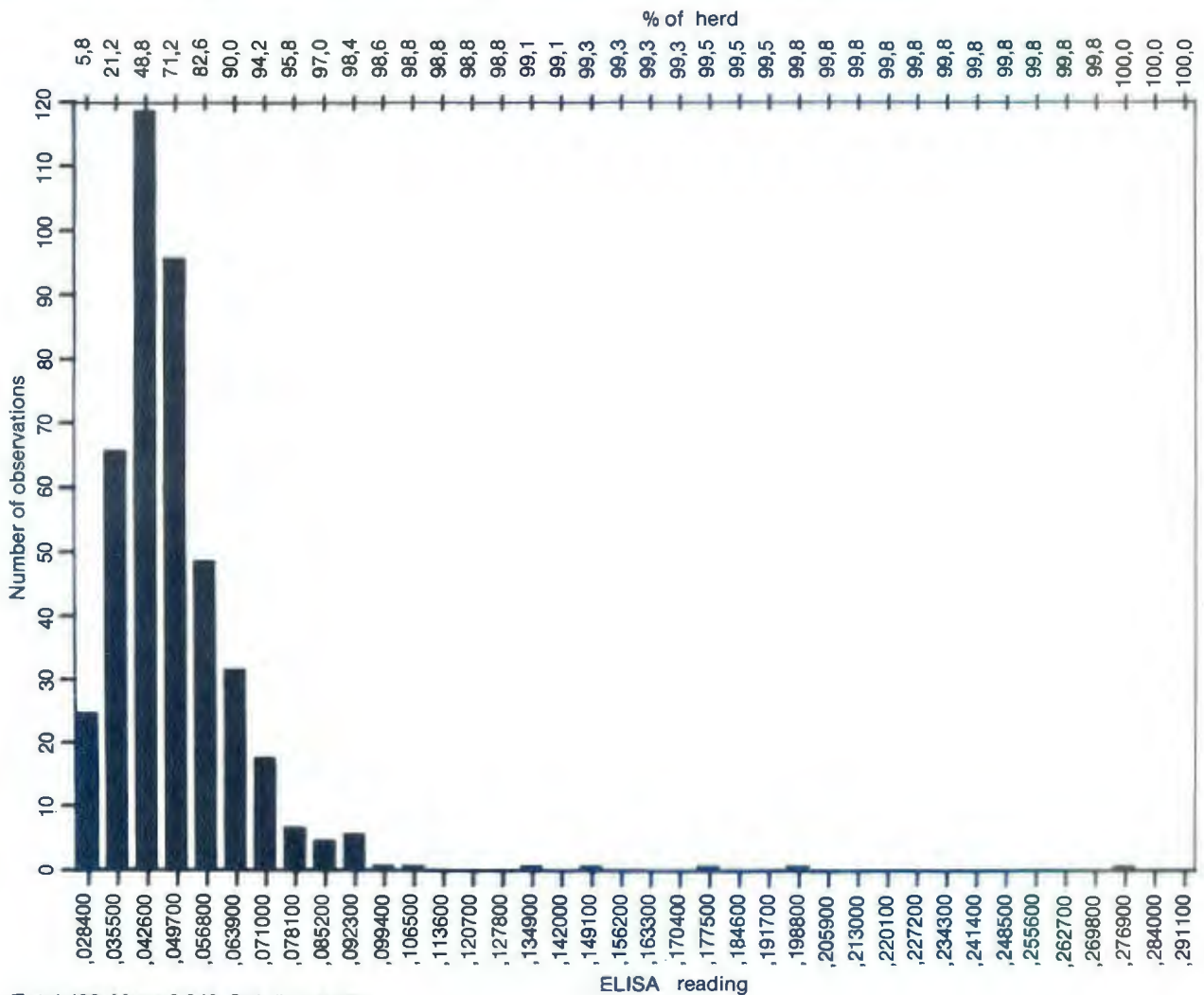


FIG. 1 ELISA data of a known negative herd

Substrate solution was prepared in a brown bottle just prior to use by adding 20 mg of orthophenylenediamine⁽⁶⁾ to 100 ml of citrate phosphate buffer. To this 10 µl of 30 % H₂O₂ was added.

Antigen plates

Each well (except those in row 1, which remained as a blanking control) of a batch of 96 well, rigid, polystyrene, non-sterile microplates⁽⁷⁾ was filled with 200 µl of a 1/4 000 dilution of antigen in coating buffer and incubated in a hot-air oven for 2 h at 37 °C. The antigen was then discarded and the plates washed 3 times in washing solution, being soaked for 3 min each time. Plates were air dried and stored at 4 °C.

Test procedure

Serum was diluted 1/1 280 in incubation buffer and 200 µl of each sample delivered into each well of the antigen plate except those in Row 1 (vide supra) and row 2, which was filled with incubation buffer as a buffer control. Positive and negative control sera were included in every plate.

The plates were incubated for 60 min at 37 °C in a hot-air oven and washed as above. Two hundred µl of conju-

gate was added to each well except those in rows 1 and 2. The plates were incubated for 60 min at 37 °C and washed. Two hundred µl of substrate was added to all the wells except those in row 1 where 200 µl of incubation buffer was used. After incubation, the plates were immediately put into the dark for 20 min, the reaction was stopped by the addition of 25 µl of 8 N H₂SO₄, and read after 10 min on a Titertek Multiskan⁽⁸⁾ using a 492 nm filter.

RESULTS

Of the 430 known negative animals, 90 % had an ELISA reading of 0,0639 and less (Fig. 1). A further 8,4 % fell within the range 0,0710 to 0,0923. The remainder were scattered, the highest reading being 0,2769.

In the positive herd, of the 91 cases having a titre of less than or equal to 15 IU/ml in the CFT; 79 had an ELISA reading of less than 0,1000, five had an ELISA reading of 0,1400 while 7 had higher readings (Fig. 2). If CFT values of greater than or equal to 36 IU/ml were considered, only 4 sera had ELISA readings of less than 0,1000. If CFT values of greater than or equal to 480 IU/ml were considered, no ELISA values of less than 0,3500 IU/ml were found. No direct correlation was found between the ELISA readings and the CFT in IU/ml.

⁽⁶⁾ Sigma Chemical Company, P.O. Box 14508, St. Louis, 63178, USA

⁽⁷⁾ Linbro 76-307-05, supplied by Seravac, P.O. Box 5933, Johannesburg 2000

⁽⁸⁾ Distributed by Seravac, P.O. Box 5933, Johannesburg 2000

CFT in IU/ml

ELISA readings	15	18-30	36-49	60-72	86-98	120-196	240-392	480-688	784
1,470									
1,400									
1,330									1
1,260									
1,190								1	2
1,120							2		4
1,050	1	1				1			4
0,980						1			4
0,910					1	1	2		2
0,840									1
0,770		1			1				4
0,700					2		1	1	1
0,630	1			2					
0,560					2		1		2
0,490								1	
0,420	1				1		1		
0,350	1					1		1	1
0,280	1	1			1		1		
0,210	2		4		4				
0,140	5	7	3	1	3				
0,070	63	14	1			2	1		
0,000	16								
Cases included	91	24	8	3	15	7	9	4	26
Mean	0,085	0,165	0,163	0,487	0,414	0,552	0,683	0,679	0,938
Std deviation	0,132	0,234	0,045	0,288	0,275	0,415	0,355	0,352	0,225

FIG. 2 ELISA readings in positive herd compared to CFT titres

The antigen remained stable at 4 °C for 4 years. It bound to the plates within 2 h and was able to detect positive animals even when diluted at 1/32 000, although the optimum dilution as 1/4 000.

DISCUSSION

If the known negative herd is considered, 94,2 % of cases had an ELISA reading of less than 0,0710. In the positive herd of the 115 animals with a CFT value of 30 IU/ml and lower, which would be considered negative (Herr *et al.*, 1982), 82,6 % had an ELISA value of 0,070 and less. For this reason animals with a reading of less than 0,0700 could usually be considered as negative. In the known negative herd, the 1,5 % of results above 0,0923 could be due to a number of reasons including vaccination with *Brucella abortus* Strain 19, technical error and damaged plates.

The lack of a direct correlation between the CFT titres of positive animals and the ELISA readings is not entirely surprising, as Butler, Feldbush, McGivern & Stewart (1978) showed that as ELISA tests are influenced by antibody affinity, the numerical values given are not a gravimetric measure of antibody in µg or mg. They suggest that data obtained with the ELISA test should be expressed in 'ELISA units'.

The results of animals in the known positive herd that had an ELISA reading of greater than 0,1000 but a CFT titre of less than 15 IU/ml should be viewed with caution. The herd history and the fact that the ELISA could perhaps be detecting types of immunoglobulins that do not fix complement must be taken into account. Further investigation into reactions of vaccinated and unvaccinated animals needs to be done.

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REFERENCES

BUTLER, J. E., FELDBUSH, T. L., MCGIVERN, P. L. & STEWART, 1978. The enzyme-linked immunosorbent assay (ELISA): A measure of antibody concentration or affinity? *Immuno-Chemistry*, 15, 131-136.

BYRD, J. W., HECK, F. C. & HIDALGO, R. J., 1979. Evaluation of the enzyme-linked immunosorbent assay for detecting *Brucella abortus* antibodies. *American Journal of Veterinary Research*, 40, 896-898.

HERR, S., ROUX, D. & PIETERSON, P. M., 1982. The reproducibility of results in bovine brucellosis serology and their correlation with the isolation of *Brucella abortus*. *Onderstepoort Journal of Veterinary Research*, 49, 79-83.

HERR, S., HUCHZERMAYER, HILDEGARD F. K. A., TE BRUGGE, LESLEY A., WILLIAMSON, CATHERINE C., ROOS, J. A. & SCHIELE, G. J., 1985. The use of a single complement fixation test technique in bovine brucellosis, Johne's disease, dourine, equine piroplasmiasis and Q-fever serology. *Onderstepoort Journal of Veterinary Research*, 52, 279-282.

LAMB, VIRGINIA L., JONES, L. M., SHURIG, G. C. & BERMAN, D. T., 1979. Enzyme-linked immunosorbent assay for bovine immunoglobulin subclass-specific response to *Brucella abortus* lipopolysaccharides. *Infection and Immunity*, 26, 240-247.

PATTERSON, J. M., DEYOE, B. C. & STONE, S. S., 1976. Identification of immunoglobulin associated with complement-fixation, agglutination and low pH buffered antigen tests for brucellosis. *American Journal of Veterinary Research*, 37, 319-324.

RAYBOULD, T. J. G. & CHANTLER, SHIRLEY, 1979. Serological differentiation between infected and vaccinated cattle by visual and quantitative immunofluorescence using *Brucella abortus* antigen coupled sepharose beads. *Journal of Immunological Methods*, 30, 37-46.

SUTHERLAND, S. S., 1985. Comparison of enzyme-linked immunosorbent assay and complement fixation test for the detection of specific antibody in cattle vaccinated and challenged with *Brucella abortus*. *Journal of Clinical Microbiology*, 22, 44-47.