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The Detection of Brucella abortus antibodies in cattle by an Enzyme Immuno Assay (EIA): Comparison between different EIA antigens, Rose Bengal and Sero Agglutination Test

Die Feststellung von Brucella abortus Antikörper bei Kühen mit der Enzym-Immun-Analyse (EIA): Ein Vergleich zwischen verschiedenen EIA-Antigenen, dem Rose Bengal-Test (RBT) und dem Serum Agglutinations Test (SAT)

by Gino Cecchini*

1 Introduction

The application of the enzyme immuno assay (EIA) technique in veterinary medicine is well documented (CHARAN GAUTAM, 1984 and VOLLER et al. 1976) and in the last few years progress has been made in improving sensitivity, specificity (SHUURS and VAN WEEM, 1977) and running time of assays (YOLKEN, 1981). The technique has high output potential (RUITEMBERG et al., 1977) is relatively easy standardized and the stability of most of the reagents (KNOOPS, personal communication, 1987) make it particularly appealing to laboratories in developing countries. The objective of this investigation was to set up an EIA for the detection of antibodies to *Brucella abortus* in cattle sera using different antigen extraction procedures and to compare the test to the Rose Bengal Test (RBT), confirming the positive findings by Sero Agglutination Test (SAT).

2 Materials and Methods

One thousand blood samples were collected from adult cattle from a State Farm in Shoa province, Ethiopia. None of the animals had ever been tested or vaccinated for Brucellosis previously. Blood was allowed to clot at 4°C, centrifuged and stored frozen until used. For technical reasons 990 samples were used.

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Conventional serologic methods:
all sera were tested using the Rose Bengal Test (RBT) (DAVIES, 1971 and BRINGLEY MORGAN et al. 1969) and all those found positive were also tested by the Serum Agglutination Test (SAT) (ALTON et al, 1975).

EIA procedure: anti bovine IgG's (Nordic Immunological Laboratories, Tilburg, The Netherlands) were labelled with horseradish peroxidase according to a two step glutharaldehide coupling procedure (ISHIKAWA et al. 1982). The tracer was further purified by gel chromatography on G-100.

96 well microplates (Immulon 1, Dynatech, USA) were coated either with soluble lipopolysaccaride phenol antigen, (batch 104, Weybridge Laboratories, UK) or with heat killed *B. abortus* cell antigen extracted according to Nielsen and Wright (NIELSEN and WRIGHT, 1984). The antigen was serially diluted starting from a concentration of 40 μ/ml in 0.06 M carbonate-biocarbonate buffer adjusted to pH 9.6. 100 μl per well of this solution was used and incubated overnight at 4°C. After incubation the plates were tapped dry and washed three times with 0.01 M PBS pH 7.2 containing 0.05% Tween 20 and incubated for a further 30 minutes with 150 μl of a 1% commercial skimmed powder milk blocking solution. Plates were washed as previously described, covered and if necessary stored frozen for further use.

100 μl of 1 to 100 PBS diluted bovine serum samples per well were incubated at room temperature for 1 hour then plates were tapped dry and washed as before. 100 μl per well of a serial dilution of the anti-bovine peroxidase labelled IgGs was added and incubated at room temperature for one hour. The plates were washed as previously mentioned and 150 μl per well of an o-phenylendyamine solution was incubated for up to 40 minutes in the dark. The reaction was stopped by adding 50 μl of a 2N sulphuric acid solution and reading was performed at 492 nm in a microplate photometer (Biorad, USA), blanked against air.

Controls: references standards were used in triplicate and were as follows: a positive control obtained from Weybridge Laboratories, a local sample consistently positive to RBT and to SAT and a negative sample to RBT and SAT. A batch of slightly positive samples to RBT and SAT (1 : 10 – 1 : 20 titre) were pooled as one sample and this was included in each assay to determine cut off point between positive and negative.

3 Results and Discussion

The optimal coating antigen concentration yielding the highest difference in optical density (OD) between a positive and a negative sample was between 1 and 5 μg/ml at 1 : 100 sample dilution.

Fraction 1 of the tracer showed optimal activity (fig. 1) and was used at 1 : 1000 dilution. The cut-off point between positives and negatives was the O. D. of the slight positive RBT and SAT pooled sample (1 : 10 – 1 : 20 titre).

Tab. 1: Results of sera positivity with ELISA compared with RBT and SAT on 990 sera.

Cow No.	EIA Extract.	EIA Crude	RBT	SAT
2122	–	+	–	–
150	–	+	–	–
1075	–	+	–	–
330	–	+	–	–
584	+	+	+	+
793	–	+	–	–
209	–	+	–	–
2412	–	–	+	–
2380	–	–	+	+
2323	±	+	+	+
448	+	–	+	+
2436	+	–	+	+
2033	+	–	+	+
2561	+	–	+	+
10	+	–	+	+
19	+	–	+	+
103	+	–	+	+
691	+	–	+	+
678	–	–	+	–
2486	+	–	+	+
2032	+	–	+	+
2564	–	+	–	–
2054	±	+	–	–
2435	–	+	–	–
2584	–	+	–	–
2607	–	+	–	–
2372	±	+	–	–
928	–	+	–	–
299		+	–	–
1054	–	+	–	–
252		+	–	–
935	±	+	–	–
1014	±	+	–	–
1074	–	+	–	–
901	–	+	–	–
329	–	+	–	–
761	–	+	–	–
675	–	+	–	–
532	–	+	–	–
40	–	+	–	–
911	–	+	–	–
793	–	+	–	–
23	–	+	–	–
429	–	+	–	–
72	–	+	–	–
16	–	+	–	–
262	–	+	–	–
42	–	+	–	–
635	–	+	–	–
252	–	+	–	–
107	–	+	–	–
236	–	+	–	–
587	–	+	–	–

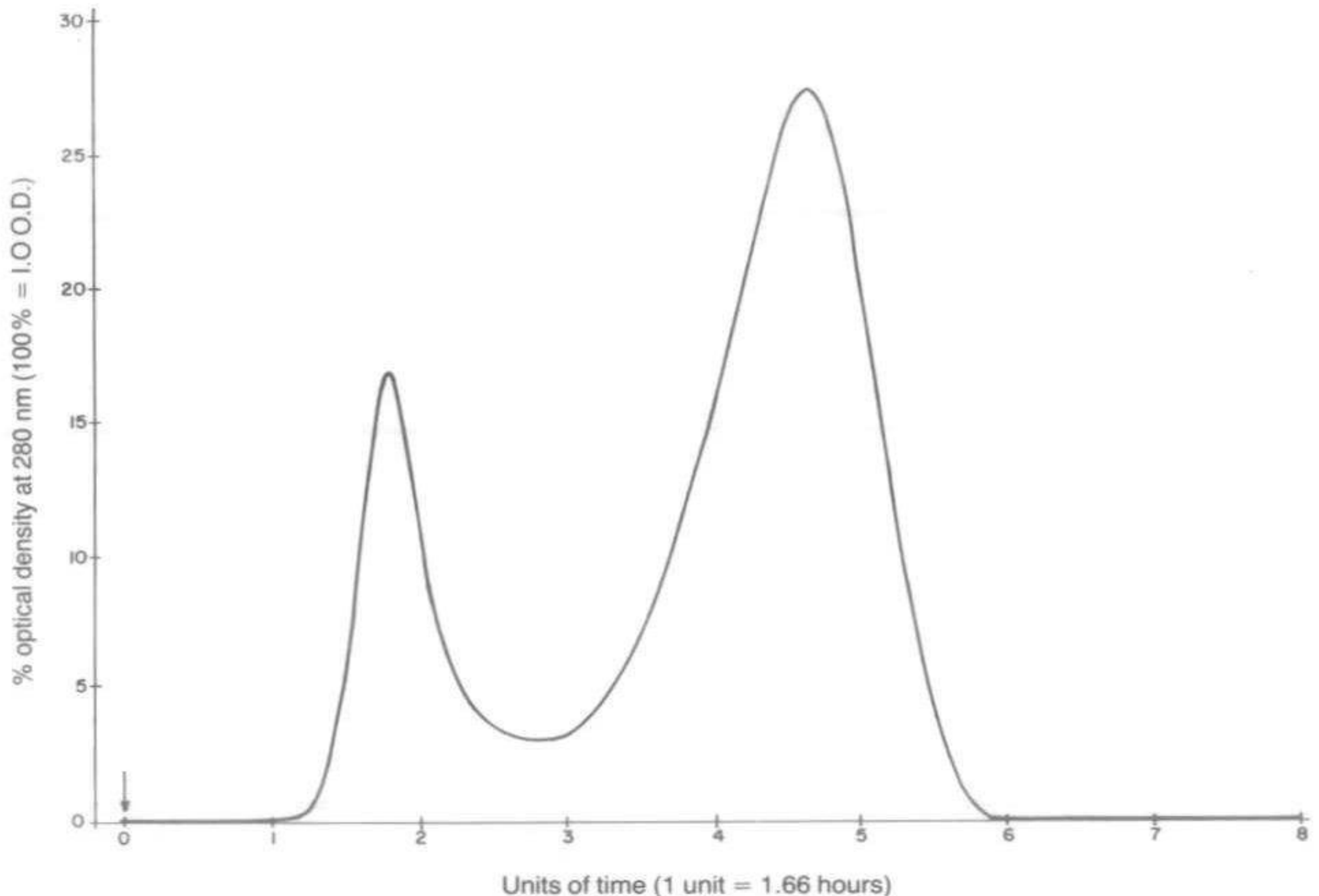

Fig.1: Rabbit anti bovine peroxidase labelled IgG's chromatographed on G-100

Of the 990 sera tested, crude and purified EIA antigens resulted in 4.9% and 0.6% respectively recorded differences if compared to RBT and SAT results (tab. 1).

Out of the 0.6%, 66.6% were slightly positives versus negative in the SAT. This could be due to both the higher sensitivity of the EIA in detecting low antibody level as well as to the inaccuracy of the visual reading ot the SAT. Results suggest that EIA can be of value in the diagnosis of *Brucella* infection for its rapidity, sensitivity and economy (2 μl of diluted sample compared to higher amounts required for both the RBT and the SAT's serial dilutions). EIA results compared favourably both to RBT and SAT using the antigen extraction method by NIELSEN and WRIGHT (1984). A high number of positives were recorded with the crude lipopolysaccharide antigen.

This might be traced back to crossreativity with other infecting organisms. The crossreactivity of *B. abortus* antigens with *Yersina enterocolitica* due to similarities in the lipopolysaccharide structure is well documented, for instance (LINDBERG et al., 1982 and HURVELL, LINDBERG, 1973). Another possible cause of the possible "false positives" with the crude antigen EIA is the low purity of the antigen used which might have contained a large amount of antigenically active bacterial cytoplasm proteins and not only the specific *Brucella* lipopolysaccharide bacterial membrane.

The visual reading of the microplates was easily performed and could be used where a microplate reader is not available. Preparation of *Brucella* lipopolysaccharide bacterial membrane.

The visual reading of the microplates was easily performed and could be used where a microplate reader is not available. Preparation of *Brucella* lipopolysaccharide antigens is simple and straightforward. In the necessity of screening large amounts of sera, accurately and cost effectively, the reported EIA using the antigen extracted according to Nielsen and Wright (NIELSEN, WRIGHT, 1984) would seem a suitable technique.

4 Summary

A microplate enzyme immuno assay (EIA) for detecting *Brucella abortus* antibodies in cattle comparing different antigen extraction methods was developed and compared with the Rose Bangal Test (RBT) and Serum Agglutination Test (SAT) on 990 cattle serum samples. Crude *Brucella abortus* antigen extracts resulted in a high number of false results (4.6%) in the EIA test. Purified antigen EIA positively correlated (0.6% discrepancy) with both RBT and SAT. The test was found to be suitable for naked eye reading.

Zusammenfassung

Es ist eine Mikroplatten-Enzym-Immun-Analyse (EIA) zur Feststellung von *Brucella abortus* bei Kühen entwickelt worden. Verschiedene Antigenextraktionsmethoden sind mit dem Rose Bengal Test (RBT) und dem Serum Aggentinations Test (SAT) bei 990 Serumproben von Kühen verglichen und getestet worden.

Unbearbeitete *Brucella abortus* Antigen-Extrakte ergaben eine hohe Rate von falschen Ergebnissen (4,6%) im EIA-Test. Gereinigte Antigen EIA korrelierten positiv (0,6% Diskrepanz) mit den beiden anderen Methoden, RBT und SAT.

Der Test ist auch zur Ablesung mit bloßem Auge durchführbar.

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