بسم الله الرحمن الرحيم

Ministry of Animal Resources & Fisheries
Animal Resources Research Corporation
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Production and Standardisation of RBPT and SAT *Brucella* antigens using Sudanese National Standard antiserum

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Introduction:

Brucellosis is a contagious disease of animals which causes enormous economical losses and serious public health hazards. It is caused by ten species of the genus *Brucella* which have variable host ranges. Diagnosis of the disease is vital for knowledge of the situation of the disease in flocks, herds and individuals are achieved by isolation of *Brucella*. However, serology is a mainstay of brucellosis diagnosis for control, eradication and export purposes.

Many serological tests are used for screening and confirmation of the disease (OIE, 2008). Sudan has about 112 million herd of livestock and standardisation of Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) antigens imported from abroad are used for screening of animal and measurement of antibody concentrations per ml in infected animals, respectively. The aim of this work was to produce locally standardised RBPT and SAT antigens using national standard antiserum equivalent to the OIEISS, to minimize costs of screening.

Material and Methods:

The RBPT and SAT were prepared using our Sudanese National standard antiserum (SNSA) as described by many investigators (Alton et al., 1975; 1988; Hendry et al., 1985; OIE, 2008).

For RBPT and SAT antigens production *B.abortus* S. 1119-3 is propagated using Roux flasks and harvested in phenol saline (Fig. 1). The cells were check for purity by examination of smears stained with Gram's stain and for dissociation by agglutination with acrifavine.

Part of the pure and smooth *Brucella* cells were stained with Rose Bengal dye, centrifuged, supernatant discarded and the diluent was added. The pH, the PCV and the optical density of the antigen were adjusted as recommended. The antigen was adjusted to agglutinate with 1/45 and not agglutinate with 1/55 dilutions of OIEISS and the Sudanese national standard antiserum prepared by Alawad and Musa (2010).

The other part of the propagated and checked cells were stirred with a magnetic stirrer for 2 hours, its pH, PCV and optical density were adjusted as recommended to match with the standard SAT antigen (Fig. 2). The product was agglutinated with different dilutions of OIEISS and the SNSA and the results were compared with those of the standard SAT antigen till it was standardised.

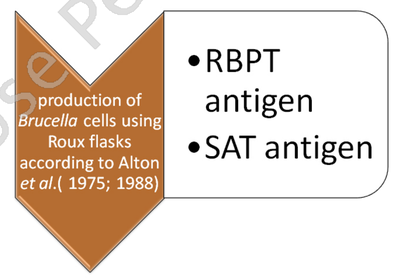

Fig. 1: RBPT and SAT preparation first step.

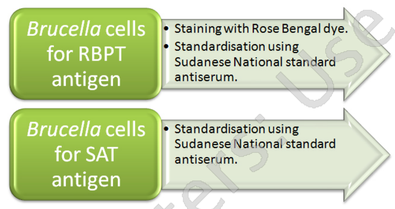

Fig. 2: Preparation of RBPT and SAT antigens major steps

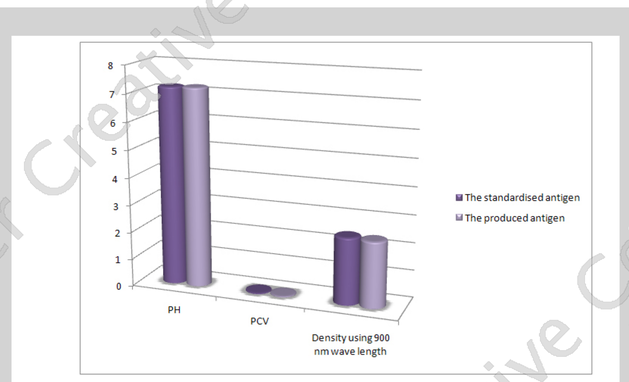

Fig.3: comparison between the standardised and produced antigen pH and densities.

Results:

The RBPT antigen produced locally was agglutinated with the 1/45 dilution of the OIEISS and the SNSA and not agglutinate at 1/55 dilutions of both antisera. The SAT antigen was agglutinated at 50% with SNSA at 1/600 to 1/1000 dilutions.

Fig.5: SAT test in macro system, using dilution series

Discussion:

Costs of imported *Brucella* antigens hamper control, eradication and export policies. Production of locally produced antigens is economically feasible (Fig.4 & 5) . The OIE provides each country with a limited number of the OIEISS. The use of the latter antigen to produce local standard antiserum is imported for harmonization of the two tests with other countries.

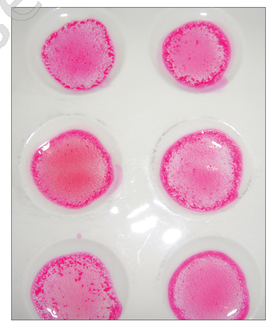

Fig.4: RBPT test Status.

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