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## Application of Brucella IgM and IgG on Buffy Coat and Serum from Population at Risk, Khartoum, Sudan

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### Abstract

Although brucellosis in human is rarely fatal, it can be severely debilitating and disabling. The infection has a tendency towards chronicity and persistence, becoming a granulomatous disease capable of affecting any organ systems.

Previous studies showed occurrence of brucellosis among population at risk in Sudan. The aim of this study was to try to apply serological tests (Rose Bengal Plate test and IgM, IgG flow assay to Buffy coat samples. Blood samples were collected from 44 persons from population at risk including livestock artificial inseminators, milkers and other farm workers in Khartoum State, Sudan. Rose Bengal Plate Test (R.B.P.T), IgM and IgG flow assay were used to analyze the samples of Buffy coat serologically. The results showed that 19 Buffy coat samples (43.2%) gave positive results with R.B. P. T; one sample (2.3%) gave positive results with IgM and 3 (6.9 %) gave positive results with IgG. In conclusion Rose Bengal Plate test and IgM, IgG flow assay can be applied to the Buffy coat; R.B.P.T. being the most reliable.

**Keywords:** (Brucellosis- farms workers-Buffy coat samples-Brucellosis in IgM-IgG).

### Introducción

Human brucellosis is one of the world most wide spread bacterial zoonotic disease and over the past decade, new foci of the disease have emerged. Every year more than 500/ 000 new cases are reported globally with annual incidence rates that varies widely from 2 to 500 per 1/000/000 population among different regions<sup>(1)</sup>.

Human brucellosis is a major debilitating zoonotic disease. It is caused by bacteria of the genus *Brucella*. The disease is endemic in the Sudan and was reported as early as 1908<sup>(2)</sup>.

Although many new diagnostic tests like automated blood culture systems and molecular methods like PCR, nucleic acid probes (etc), have been developed for diagnosis of brucellosis, they are quite expensive and need sophisticated equipments and skilled expertise. As this is not possible for all testing laboratories, simple, cost effective, reliable and reproducible methods need

to be developed and tested<sup>(3)</sup>. In addition the usual serological tests may fail to detect infection with *Brucella canis*<sup>(4)</sup>.

The objective of this study was to apply Rose Bengal Plate Test, IgM and IgG flow assay on Buffy coat samples to investigate their reliability.

### Materials and Methods

#### Study Area

The study was carried out on population at risk in Khartoum state, Sudan. The population, on their consent, included artificial inseminators, milkers, veterinarians and other farm workers.

#### Sampling Methods

Blood samples for serological examinations were collected from 44 persons in a population at risk, Khartoum state, Sudan including artificial inseminators, milkers, veterinarians and other farm workers after obtaining their consent.

Two and half ml of blood were collected and kept in 2.5 EDTA container, and used to obtain Buffy coat by centrifugating at 6000 rpm for 30 min to separate the Buffy coat. Then the samples were re-centrifuged using ultra centrifuge at 15,000 rpm for 5 and 15min consecutively to separate the Buffy coat<sup>(5)</sup>.

### **Rose Bengal Plate Test**

The antigen used in the Rose Bengal Plate Test (RBPT) was obtained from Sudanese Veterinary Research Laboratory.

### **The Test Procedure**

The Buffy coat samples were removed from deep freezer and kept overnight at 4° C and then the antigen and serum were removed from the refrigerator and placed at room temperature till brought to room temperature and then the test was performed following the procedure described by Ferreira, et al <sup>(6)</sup>.

The test was done by dispensing 0.025 ml of each Buffy coat to be tested to an enamel plate. The same amount of Rose Bengal was added to each Buffy coat and both were mixed together, rocked by hand for four minutes after which the test was immediately read. Any sort of agglutination appeared was considered as positive.

### **IgM and IgG Flow Assay**

The kits were obtained from the Royal Tropical Institute (Netherlands) and stored at 4 C° till used. The test was performed to all Buffy coat samples obtained from the fields. The standard assay procedure for serum was followed as accompanied with the kit protocol.

### **Interpretation of Test Results**

A positive result was indicated by the presence of a line at the test zone and line at the control zone and the degree of the positive sample was determined after comparing with the protocol provided with the kits.

### **Ethical Consideration**

The study proposal was approved by the ethical committee of Alzaiem Alazhari University. The subject was informed about the study before samples collection. Positive cases were given free medication.

### **Results**

From a total of 44 samples of Buffy coat collected from the sample population at risk 19 Buffy coat (43.2%) gave positive results with R.B. P. T; one sample (2.3%) gave positive results with IgM and 3 ( 6.9 % ) gave positive results with IgG. In conclusion Rose Bengal Plate test and IgM, IgG flow assay can be applied to the Buffy coat; R.B.P.T. being the most reliable.

### **Discussion**

R.B.P.T, IgM and IgG serological tests can be applied on Buffy coat. IgG gave more positive results than IgM.

I study in western Sudan, in humans associated with live stock, there were 13% positive using Rose Bengal plate test and SAT <sup>(7)</sup>, while only 1% was detected in occupation contacts in human in eastern Sudan, using slide agglutination test and positive confirmed by tube agglutination test <sup>(8)</sup>. These were less than what was found in this study using R.B.P.T (43.1%).

In Khartoum in 2007, 40% from the examined milkers' sera showed positive agglutination using standard tube agglutination test these findings were similar to the results of this study. <sup>(9)</sup>

### **Conclusion**

R.B.P.T, IgM and IgG serological tests can be applied on Buffy coat; R.B.P.T. being the most reliable.

### **Acknowledgment**

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IgM FPOW ASSAY KITS. Thanks also due to the farms workers in this study.

### Limitation of the Study

Due to the lack of published data about use of serological tests on buffy coat, the samples were few.

Also due to difficulty in obtaining samples, and tests kits from Netherlands as they are not available in locally.

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