

**ORIGINAL ARTICLE**

**Seroprevalence of Brucellosis Associated with Abortion among Sudanese Pregnant Women attending Omdurman Maternity Hospital, Sudan**

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**Abstract:**

**Background:** Human Brucellosis remains the most common zoonotic disease endemic in the Mediterranean area, Middle East, Southwest Asia, and parts of Latin America. The disease is endemic in the Sudan and was reported as early as 1908. It is a commonly misdiagnosed febrile disease with malaria and typhoid fever. Brucellosis is a multi-systemic disease characterized with fever, osteoarticular involvement, neuropathies, chorea, and meningoencephalitis, gastrointestinal, hepatobiliary, genitourinary, musculoskeletal, cardiovascular, pleural effusion and pneumonia. Due to increase rates of spontaneous abortion, premature delivery and intrauterine infection with fetal death caused by this disease, there is need for urgent intervention to reduce maternal mortality rate and increase child survival. This study was designed to investigate the seroprevalence of Brucellosis associated abortion among Sudanese.

**Objective of the Study:** To investigate the seroprevalence of Brucellosis associated abortion among Sudanese attending Omdurman Maternity Hospital, Sudan.

**Materials and Methods:** A cross-sectional study was carried out among 120 pregnant women who have aborted and 21 pregnant women who have not aborted

who attended Omdurman Maternity Hospital during the period from August to October (2015). Serum samples were collected to detect the presence of the Brucella anti bodies (BAbs) using Rose Bengal plate Test (RBPT) as screening test and Serum Agglutination Test (SAT) as confirmatory test. A structured questionnaires was designed to record demographical clinical data.

**Results:** Prevalence of Brucella disease was found to be 1.7%(2/120) in pregnant who have aborted and increased significantly with milk consumption, cheese eating, and yoghurt consumption.

**Conclusion:** These findings demonstrated the prevalence of Brucellosis associated abortion among Sudanese women, so that the coordination between medics and researchers started in this field.

**Keywords:** RBPT, SAT, Aborted women, pregnant have not aborted, Sudan

## **Introduction:**

Human Brucellosis remains the most common zoonotic disease worldwide, with more than 500 000 new cases annually <sup>(1)</sup>. Although Brucellosis has been eradicated in many developed countries, it is still endemic in the Mediterranean area, Middle East, Southwest Asia, and parts of Latin America. In a recent study in the United Kingdom(UK)<sup>(2)</sup>, only two of 438 pregnant women exhibited Brucella antibodies(BABs), both patients originating from Sudan. But In Sudan, the disease was reported as early as 1904<sup>(3)</sup>. Also Simposon <sup>(4)</sup> reported 20 clinically diagnosed cases of Brucellosis in the Blue Nile and Kassala provinces. Then Bennet<sup>(5)</sup> isolated *Brucella abortus* for the first time from a dairy herd in Khartoum. Furthermore Hasseeb <sup>(3)</sup> who was the first to confirm a case of human Brucellosis. In addition to that Hasseeb<sup>(3)</sup> and Daffalla <sup>(6)</sup> stated that the disease was diagnosed in all provinces except Bahr El Gazal, in the Southern Sudan up to 1955.

The disease is endemic in the Sudan and was reported as early as 1908 <sup>(6)</sup>. In spite of this, it is commonly misdiagnosed as another febrile disease <sup>(7)</sup>. Malaria and typhoid fever are the commonest diseases for which Brucellosis is misdiagnosed<sup>(8)</sup>. The source of any human case is an animal directly or indirectly through contaminated raw products from infected animals<sup>(7)</sup>. The definitive diagnosis of human Brucellosis depends on isolation of *Brucella species* from cultured human specimen. However, these bacteria are slowly growing

microorganisms and few number cells commonly in the culture yields no growth. Even if they grow, they are highly infectious to the laboratory personnel <sup>(7)</sup>. For these reasons, serologic diagnosis has been adopted. On the other hand the brucellosis is acquired either by contact with infected animals or consumption of contaminated milk or dairy products. The disease has major economic impact not only due to time lost by patients from normal daily activities but also due to the loss in animal husbandry <sup>(9)</sup>. Indeed, Brucellosis is characterized by abortion and loss of fertility in farm animals <sup>(10)</sup>. In humans, the symptomatology is not specific, and this makes the diagnosis quite challenging. Patients may present with an intermittent fever, joint pain (including arthritis), neurologic manifestations, and so forth <sup>(11)</sup>. The association between abortion/stillbirth and Brucellosis in humans is controversial <sup>(12)</sup>. This could be due to the absence of erythritol (a 4-carbon sugar alcohol which is the preferred carbon source for *Brucella*) in human placenta as opposed to ruminant placenta <sup>(13)</sup>. However, a recent study has demonstrated that *Brucella* replicates in several human trophoblast subpopulations and can interfere with the invasive capacity of extravillous trophoblast-like cells *in vitro* <sup>(14)</sup>. Studies investigating an association between human Brucellosis and abortion *in vivo* are scarce.

In humans, Brucellosis is a multi-systemic disease. Fever is the most common feature, followed by osteoarticular involvement (Sacroiliitis, spondylitis, peripheral arthritis, and osteomyelitis) <sup>(14)</sup>. However, all systems might be affected, such as the nervous (neuropathies, chorea, and meningoencephalitis), gastrointestinal, hepatobiliary (hepatomegaly), genitourinary (Orchiepididymitis, glomerulonephritis and renal abscesses), musculoskeletal, cardiovascular (endocarditis, mainly with involvement of the aortic valve) and pulmonary (pleural effusions and pneumonia) systems. Approximately 10% of patients with brucellosis experience relapses, 90% of which occur within a year after discontinuation of antimicrobial drug therapy <sup>(15)</sup>. Disease reactivation has been described as long as 28 years after the initial infection <sup>(16)</sup>. Increased rates of spontaneous abortion, premature delivery and intrauterine infection with fetal death have been described among pregnant women with clinical evidence of brucellosis <sup>(17)</sup>.

Brucellosis is routinely misdiagnosed, or at best diagnosed incidentally; therefore, physicians in both endemic and non-endemic areas should be aware of Brucellosis and consider this disease in the differential diagnosis of febrile episodes during

pregnancy. Blood culture is the reference standard for the diagnosis of *Brucella* infection in humans <sup>(18)</sup>. The rose Bengal plate test is used as a screening serological test, and positive results are confirmed by the serum agglutination test <sup>(18)</sup>. Other tests, such as ELISA(Enzyme linked immune assay and PCR (Polymerase chain reaction), are increasingly used for the diagnosis of Brucellosis.

### **Methodology:**

This is a descriptive, analytical- cross sectional study. The study was carried out at Omdurman Maternity Hospital which includes eight operation rooms. The total number of beds in the hospital are 187 beds, 37 beds from gynecology rooms. Pregnant women complicated with abortion who attended Omdurman Maternity hospital were included, Pregnant women diagnose as abortion who were treated with anti-biotics and refused to participate in the study were excluded. The aborted women were informed about the purpose of the study before collection of the specimens, and verbal consents were taken. Personal and clinical data were obtained by direct interviewing questionnaire from the aborted women. Firstly the venous blood sample was aseptically collected from disinfected venous area by using 5ml sterile syringe from the pregnant women after the moment of the abortion and then was put in sterile plain container which was labeled with patients name, date of sample collection, hospital name and serial number and the specimen was left for clotting. After the clotting the venous blood sample was separated to serum by using centrifugation (3000 rpm for 5 minutes) and then the serum was stored frozen at -20 ° C even in (Brucella Department – Veterinary Research Institute – Animal Resources Research Corporation – Ministry of Livestock, Fishery and Range Land – Sudan). In analysis: The rose Bengal plate test was used as a screening serological test, and positive results were confirmed by the serum agglutination test.

### **Statistics**

Collected data were tabulated and analyzed using the statistical package of social science (SPSS) program version19. Results were considered significant if p value was  $\leq 0.05$  and Chi – square statistical analysis was used to determine p value significance range.

**Results:**

**Detection of Brucellosis in pregnant women associated with abortion when used Rose Bengal PlateTest (RBPT):**

In this study, 120 serum biological samples were obtained from pregnant women with abortion who attended Omdurman Maternity Hospital. Two serum samples from 120 samples were positive (1.7%) for Brucella anti-bodies Table(1), while the remaining serum samples (118) samples were negative (98.3%) Table (1) when used Rose Bengal test.

**Table (1) : Frequencies of positive and negative Brucellosis results using Rose Bengal Test pregnant women with abortion.**

RBPT result	Frequency	Percent
Positive	2	1.7%
Negative	118	98.3%
Total	120	100.0%

The age distribution of seropositive and seronegative for Brucellosis ranged from less than 18 to 50 years. The seropositive result was 0.8% among Brucellosis patients in 18-28 year ages and 29-39 year ages respectively, followed by seronegative result was 0.0% in <18 year age and 40-50 year age respectively.

**Table (2) : Distribution of age groups among positive and negative Rose Bengal Test Results women with abortion**

Age group	RBT result frequency			% of frequency		Total percentage
	Positive	Negative	Total	Positive	Negative	
<18	0	1	1	0.0%	0.8%	0.8%
18 – 28	1	64	65	0.8%	53.3%	54.2%
29 – 39	1	48	49	0.8%	40.0%	40.8%
40 - 50	0	5	5	0.0%	4.2%	4.2%
<b>Total</b>	<b>2</b>	<b>118</b>	<b>120</b>	<b>1.7%</b>	<b>98.3%</b>	<b>100.0%</b>

**(P value = .985)**

The residence distribution of seropositive was in women that living in Khartoum

state and seronegative was in others women that lived in others states.

**Table (3) : Distribution of residence among positive and negative Rose Bengal Test Results in aborted women**

Residence	RBT result frequency			% of frequency		
	Positive	Negative	Total	Positive	Negative	Total percentage
Khartoum	2	112	114	1.7%	93.3%	95.0%
Algazera	0	1	1	0.0%	0.8%	0.8%
White Nile	0	3	3	0.0%	2.5%	2.5%
Kordofan	0	1	1	0.0%	0.8%	0.8%
Naharalneel	0	1	1	0.0%	0.8%	0.8%
Total	2	118	120	1.7%	98.3%	100.0%

(P value = .999)

The occupation distribution of seropositive was in house keeper and seronegative was in others occupations.

**Table (4) : Association of positive and negative Rose Bengal Results with Occupation in aborted women**

Occupation	RBT result frequency			% of frequency		
	Positive	Negative	Total	Positive	Negative	Total percentage
house keeper	2	95	97	1.7%	79.2%	80.8%
Teacher	0	8	8	0.0%	6.7%	6.7%
Veternirian	0	1	1	0.0%	0.8%	0.8%
Nurse	0	2	2	0.0%	1.7%	1.7%
student	0	4	4	0.0%	3.3%	3.3%
free worker	0	2	2	0.0%	1.7%	1.7%
nutritional guider	0	1	1	0.0%	0.8%	0.8%
wire engineer	0	1	1	0.0%	0.8%	0.8%
laboratory technician	0	1	1	0.0%	0.8%	0.8%
laboratory helper	0	1	1	0.0%	0.8%	0.8%
police helper	0	1	1	0.0%	0.8%	0.8%
cleanity	0	1	1	0.0%	0.8%	0.8%

company						
Total	2	118	120	1.7%	98.3%	100.0%
( P value =1.000)						

The Level of study distribution of seropositive was in secondary level and seronegative was in others level .

**Table (5) : Association of positive and negative Rose Bengal Results with Level of education in aborted women**

Level of study	RBT result frequency			% of frequency		Total percentage
	Positive	Negative	Total	Positive	Negative	
Illiterate	0	10	10	0.0%	8.3%	8.3%
Basic	0	40	40	0.0%	33.3%	33.3%
Secondary	2	38	40	1.7%	31.7%	33.3%
Under graduate	0	3	3	0.0%	2.5%	2.5%
graduate	0	25	25	0.0%	20.8%	20.8%
post graduate	0	2	2	0.0%	1.7%	1.7%
Total	2	118	120	1.7%	98.3%	100.0%
(P value = .540)						

**Table (6) : Association of positive and negative Rose Bengal Results with the number of past abortion in aborted women**

Other number of last abortion	RBT result frequency			% of frequency		Total percentage
	Positive	Negative	Total	Positive	Negative	
One	0	20	20	0.0%	16.7%	16.7%
Two	2	32	34	1.7%	26.7%	28.4%
Three	0	19	19	0.0%	15.8%	15.8%
Four	0	18	18	0.0%	15.0%	15.0%
Five	0	16	16	0.0%	13.3%	13.3%
Sex	0	4	4	0.0%	3.3%	3.3%
Seven	0	4	4	0.0%	3.3%	3.3%
Eight	0	2	2	0.0%	1.7%	1.7%

Nine	0	3	3	0.0%	2.5%	2.5%
Total	2	118	120	1.7%	98.3%	100.0%
(P value = .742)						

**Table (7) : Association of positive and negative Rose Bengal Results with the gestational age (trimester) in aborted women**

Trimester	RBT result frequency			% of frequency		Total percentage
	Positive	Negative	Total	Positive	Negative	
First Trimester	1	68	69	0.8%	56.7%	57.5%
Second Trimester	1	37	38	.83%	30.8%	31.7%
Third Trimester	0	13	13	0.0%	10.8%	10.8%
Total	2	118	120	1.7%	98.3%	100.0%
(P value = .796)						

**Table (8) : Association of positive and negative Rose Bengal Results with history of contact with animals in aborted women**

Animals	RBT result frequency			% of frequency		Total percentage
	Positive	Negative	Total	Positive	Negative	
Yes	0	21	21	0.0%	17.5%	17.5%
No	2	97	99	1.7%	80.8%	82.5%
Total	2	118	120	1.7%	98.3%	100.0%
(P value = .511)						

The type of animals distribution of seropositive was in people that had no animals and seronegative was in others people that had animals. Table (8) shows that the 2 seropositive women were not in contact with animals and seronegative women did not have contact with animals.

**Table (9) : Association of positive and negative Rose Bengal Results with Type of animals contacted in aborted women**

Type of animals	RBT result frequency			% of frequency		
	Positive	Negative	Total	Positive	Negative	Total percentage
Goats	0	1	1	0.0%	0.8%	0.8%
Sheep	0	1	1	0.0%	0.8%	0.8%
Cows	0	1	1	0.0%	0.8%	0.8%
Cats	0	8	8	0.0%	6.7%	6.7%
Goats and Sheep	0	4	4	0.0%	3.3%	3.3%
Goats, Sheep and Camels	0	1	1		0.8%	0.8%
Dogs	0	2	2	0.0%	1.7%	1.7%
Sheep and Cows	0	1	1	0.0%	0.8%	0.8%
None	2	99	101	1.7%	82.5%	84.2%
Total	2	118	120	1.7%	98.3%	100.0%

(P value = 1.000)

The milk drinking distribution of seropositive was in people that drinking milk and seronegative was in others that had no relationship with milk drinking. Table (9) shows that the 2 seropositive women were drinking milk and seronegative women were both drinking milk (96) and not drinking milk (22).

**Table (10) : Association of positive and negative Rose Bengal Results with Milk drinking in aborted women**

Milk drinking	RBT result frequency			% of frequency		
	Positive	Negative	Total	Positive	Negative	Total percentage
Yes	2	96	98	1.7%	80.0%	81.7%
No	0	22	22	0.0%	18.3%	18.3%
Total	2	118	120	1.7%	98.3%	100.0%

(P value = .499)

The cheese eating distribution of seropositive was in women who were eating

cheese and seronegative was in women who were eating cheese (101) and not eating cheese (17).

**Table (11) : Association of positive and negative Rose Bengal Results with Eating cheese in aborted women**

cheese eating	RBT result frequency			% of frequency		
	Positive	Negative	Total	Positive	Negative	Total percentage
Yes	2	101	103	1.7%	84.2%	85.8%
No	0	17	17	0.0%	14.2%	14.2%
Total	2	118	120	1.7%	98.3%	100.0%

(P value = .562)

**Table (12) : Association of positive and negative Rose Bengal Results with Yoghurt eating in aborted women**

Yoghurt eating	RBT result frequency			% of frequency		
	Positive	Negative	Total	Positive	Negative	Total percentage
Yes	2	104	106	1.7%	86.7%	88.3%
No	0	14	14	0.0%	11.7%	11.7%
Total	2	118	120	1.7%	98.3%	100.0%

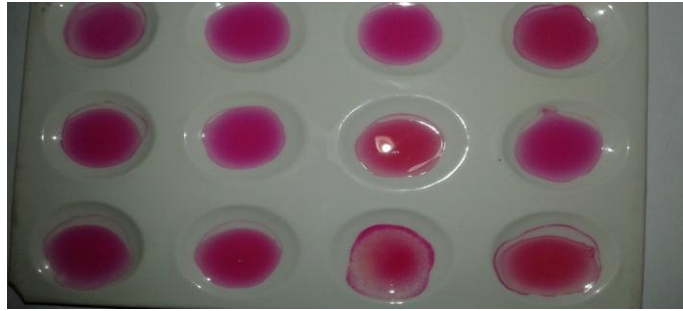
(P value = .604)

**Confirmation of Brucellosis positive patients when used Serum Agglutination Test (SAT) :**

When the positive results of rose Bengal plate test was confirmed with serum tube agglutination test that it gave negative results.

**Table( 13)Frequency of Negative Brucellosis Result when used Serum Tube Agglutination Test as confirmatory test according to the study of pregnant women associated with abortion.**

STAT result	Frequency	Percent
Negative	120	100.0%
Total	120	100.0%



**Figure (1) : Image has shown positive and negative Rose Bengal Plate Test results Sensitivity, Specificity, Odd ratio, and Relative risk of Rose Bengal test among Brucellosis patients:**

The data analysis by using Fisher's exact test revealed significant variation between both control and infected patients as  $p$  value=0.0046. the sensitivity of the test was 0.01667 which revealed on 95% confidence interval that ranged between 0.002025 to 0.05891 while the specificity was 0.8095 which revealed more reliable value on 95% confidence interval that ranged between 0.5809 to 0.9455 so that the rose Bengal test was low sensitive but had high specificity and odd ratio was 0.07203 with 95% confidence interval between 0.01224 to 0.4239 which indicated that exposure negatively related to disease and relative risk factor was 0.3814 with 95% confidence interval between 0.1227 to 1.18, both of them <1. Furthermore the positive predictive value was 0.3333 which revealed on 95% confidence interval that ranged between 0.04327 to 0.7772 and negative predictive value was 0.1259 which revealed on 95% confidence interval that ranged between 0.07510 to 0.1939.

Data analyzed	Aborted women	Control	Total
Positive RBT	2	4	6
Negative RBT	118	17	135
Total	120	21	141

**Discussion :**

Diagnosis of Brucellosis by isolation of the organisms by culture is the gold standard, but it is difficult, hazardous, time consuming, lacks sensitivity to patients with chronic disease, and moreover, it requires highly skilled personnel

and special level III bio safety cabinets. So, the diagnosis is based on epidemiological data, clinical findings and serological laboratory tests<sup>(19)</sup>. It is recommended that no single serological test is satisfactory for diagnosis of brucellosis and that combined tests can be used<sup>(20)</sup>. The Rose Bengal Plate Test is an agglutination screening test that detects the IgG1 fraction of antibodies and is cheap, simple, highly sensitive, but of low specificity. The Serum Agglutination Test is another agglutination test that is used for the detection of IgM and IgG antibodies and is mostly positive in the acute stage of the disease, but the test is less sensitive in the sub-acute and chronic stage<sup>(20)</sup>.

In this study the detection of Brucellosis in aborted women was (1.7%) from total specimens which were 120 samples, when used the serological test the rose Bengal plate test, and the positive cases were due to the closed relationship with milk consumption, cheese eating (made under aseptic technique), and also yoghurt consumption, so that this interpretation in recent analysis of database embedded the association of abortion with brucellosis through its detection serologically with rose Bengal plate test that showed two positive results of serum samples and the remained serum samples from aborted women (118) samples were negative(98.3%), Therefore, the positive result of rose Bengal plate test gave inverse result when confirmed with serum agglutination test, because the serum agglutination test may be negative in the early stages of infection and in long standing chronic infections<sup>(21)</sup> and this interpretation supported the current study. The negative results of rose Bengal test, interpreted that the abortion must be due to other microorganisms such as Cytomegalo virus, rubella virus, toxoplasma gondii, and herpes simplex virus type one and two can cause the abortion. And also must be due to other autoimmune diseases which have the role in the abortion. False positive results probably due to cross reaction with other bacteria as E.coli, Salmonella, Yersinia, and enterocoli<sup>(19)</sup> and according to literature there were no published studies in Sudan to compare with recent study, thus this study was the first Brucellosis seroprevalence study among aborted women in the Sudan. On the other hand there are no more documented data published in this topic internationally, therefore the positive results of rose Bengal test agreed with Abushehada<sup>(22)</sup> in Jordan, where the true seroprevalence among women with miscarriage was 2% from 9 Serum samples collected from 445 women with miscarriage in Jordan. However the current seropositive disagreed with Nassaji<sup>(23)</sup>, where 81 women with spontaneous abortion were 6.2% , because he analyzed

by enzyme linked immune assay and disagreed with Nadine <sup>(24)</sup>, where a total of 60 women were recruited and 15 (25%) were Brucella seropositive; indicated that consumption of raw cow's milk, followed by contact with farm animals, was the main risk factor associated with Brucella seropositivity and my positive findings disagreed with Kassiri <sup>(25)</sup>, from a study conducted in Iran, where 79% of infected patients contracted the disease via consumption of unpasteurized dairy products while 21% had a history of animal contact and moreover, after comparing the positive results with potentially confounding variables, such as age, residence, occupation, study level, blood group, rhesus factor, pregnancy times, Brucellosis treatment, abortion, abortion times, trimester, animals presence, animals types, drinking of milk, eating of cheese, and consumption of yoghurt, presented that inverse relationship between these variables and positive result, and there were no significance between those variables with positive results when determined p value ( $\leq 0.05$ ). Finally when evaluated the sensitivity and specificity of rose Bengal plate test, the rose Bengal test gave low sensitivity and high specificity, the interpretation of this study agreed with Chernysheva <sup>(26)</sup> who said that the rose Bengal test has low sensitivity particularly in long evolution (chronic) cases and Konstantinidis <sup>(27)</sup> who said relatively with low specificity in endemic areas.

## **Conclusion:**

The febrile conditions among pregnant women are alarming, so that the coordination between medicals and researches was started in this field. Brucellosis was found to be contributory factor in women with abortion in Omdurman Maternity hospital.

## **Ethics**

The permission to conduct this study was obtained from College of Graduate Studies, Sudan Academy of Sciences and verbal consent was obtained from aborted women and head of gynecology unit of Omdurman maternity hospital.

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