Seroprevalence of brucellosis among Blood donors in Khartoum State

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ABSTRACT

This study was conducted during the period from March to May 2014 . The aim of this study was to determine the seroprevelance of human brucellosis amoge the blood donors, in Khartoum State, Sudan.

A total of one hundred and fifty volunteer participant (n=150) were included in the study. The diagnosis of human brucellosis was based on Rose Bengal plate Test (RBpt) to screen the prevalence of anti_brucella antibodies and Standard Agglutination Test (SAT) to determine the titer of *B.abortus* and *B.meltiensis*

Males samples number was 133(88.7%) , whereas females samples were 17(11.3%). Twenty three(15.3%) out of 150 donors showed positive result for *Brucella*.

Twenty (13.3%) samples from males were positive, female positive samples were 3(2%). The prevalence of *Brucella* species from blood donors, *B.abortus* seropositive was 21(70%) whereas *B.meltiensis* seropositive was 9(30%) .

Seven samples were positivefor both *B.abortus* and *B.meltiensis* all from males.

Brucella abortus antibodies were detected in 20(66.7%) males and in one(3.3%) females , while *Brucella meltiensis* antibodies were detected 7(23.3%) in males and 2(6.7%) in females .

Key words : *Brocella* , blood bank ,seroprevalence

INTRODUCTION

Animal and human health's are inextricably linked. People depend on animals for nutrition, socioeconomic development and companionship. Yet animals can transmit many diseases to humans. Diseases transmitted from animals to humans are termed zoonosis. Some of them are potentially devastating.

Brucellosis is the zoonosis of worldwide distribution and common cause of economic losses and ill health among animals and human populations (Bennet ., 1943).

Brucellosis is an infectious zoonotic disease caused by bacteria of the genus *Brucella*. It is primarily a disease of domestic animals. Various *BrucelIa* species affect sheep, goats, cattle, deer, elk, pigs, dogs and several other vertebrates. Humans become infected by coming in contact with infected animals or animal products (mostely raw milk and its derivitis). Only in exceptional circumstances transmission can be from man to man (e.g., unsuspected brucellosis in blood donors may lead to infection of recipients, or from lady to her child during breast-feeding). Occasionally, brucellosis is acquired directly from contaminated

laboratory materials (Zammit, 1984).

Some vaccines were used in livestock, most notably was *B. abortus* strain 19, which may cause disease in human if accidently infected. The disease in humans can cause a range of symptoms like those associated with many other febrile diseases, but with emphasis on muscular pain and sweating. Severe infections of the central nervous system or lining of the heart may occur. Brucellosis also cause long-lasting or chronic symptoms that include reclessent fevers, joint pains, orchitis, meningitis and fatigue (CDC, 2005).In human mortality is negligible, but the illness can last for several years (Madkour.,2001). In animals, brucellosis mainly affects reproduction and fertility, reduces survival of newborns and reduces milk yield, but mortality adult animals is insignificant (Sewell and Brocklesby., 1990). *Brucella* species known are *Brulcella abortus* cattlearethemain reservoir), *B. suis* (pigs, hares, rodents and reindeers), *B. canis* (dogs), *B. avis* (sheep), *B. neotomae* (wood rat neotomae lepida Thomas). *Brulcella* species have also been isolated from several marine mammal species; *B. cetaceae*" preferentially infecting cetaceans" and *B. pinnipediae* " preferentially infecting pinnipeds" (Cloeckaert and Zygmunt .,2001). In all host species brucella grow intercellularly producing a variable bacteraemic phases followed by localization in the tissues o the genital tract and in the mammary glands and in humans mainly in the reticuloendothelial system (Jinkyung and Gary, 2003). Pathological manifestations as abortion, placentitis, endometerities, orchaitis, hygromas, epidedymitis and arthritis are not uncommon sequellae of infection with brucellosis in animals (Blood *et al*., 1983). Bruccellosis is diagnosed either by isolation of Brucella organism organism or by a combination of serological tests and clinical findings consistent with brucellosis (AI Sekait ., 1999). The main way of preventing brucellosis is by control of animal brucellosis by using vaccines, also by using fastidious hygiene in producing raw milk products. In many developed countries, the animals diseases have been brought under control, which has led to

subsequent decrease in the number of human cases(WHO.,2008).

Brulcella was isolated in 1886 by David Bruce, an army doctor serving with the British army on the island of Malta. He isolated the organisms from the spleen of four British soldiers dying on the island of Malta from a disease known as Malta fever, or Mediterranean remittent fever (Greenwood *et al*., 2000).

At that time, the disease had a high prevalence among the army and navy personnel and among the islands civilian population.

The name *Brulcella* was subsequently given in honour of Bruce who established it as the cause of the disease in experimental monkeys (Greenwood *et al.*, 2000).

Human brucellosis in Sudan:

Nomads and occupational, namely veterinary staff, abattoir and butchers-house workers were found to be most affected with the disease (Mus a, 1995). The disease was diagnosed in humans in Berber in the Sudan since 1904 (Haseeb.,1950). In 1908, Bousefield reported a case of Malta fever. In the same year 20 cases were reported (Simpson.,1908), 19 of which were clinically diagnosed at Roseires (Blue Nile Province) and one at Kassala. The data given by (Haseeb.,1950) between the year 1925 and 1942 gave a total record of 920 human cases with occurrence in everyone of the eighteen provinces. Medical reports between the year 1928 and 1937 showed the occurrence of 311 human cases and the distribution of the disease was reported from all the nine provinces of the Sudan (Dafaalla.,1962). The organism was isolated from man (Erawa.,1966). In 1982, the Sudan medical reports documented a 242 cases of human brucellosis. In 1994, AL- Sharif obtained positive results from abattoir workers in Umdurman city slaughter house. In a country where hospital services, particularly where animal abide, is scarce and where fever is "just. a fever" its highly likely that the difference between the actual incidence and the recorded one may be highly significant (Adil.,2007).

The disease in humans:

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are

apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as many signs referable to almost

any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized

infection or a non-specific syndrome resembling the "chronic fatigue syndrome". The disease is always caused by infection with a *Brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens. (Ariza *et al.,* 2007)

Evidence in support of the diagnosis includes:

• A history of recent exposure to a known or probable source of *Brucella* spp.

This includes common host species, especially cattle, sheep, goats, pigs, camels, yaks, buffaloes or dogs; consumption of raw or inadequately cooked milk or milk products, and, to a lesser extent, meat and offal derived from these animals. In addition, the resistance of the organism and its high infectivity make environmental contamination a probable hazard, although this is always difficult to prove. Occupational exposure and/or residence in an area in which the infection is prevalent, also raise the probability of the diagnosis. (Ariza *et al.,* 2007)

• Isolation of *Brucella* spp. from the patient.

• Demonstration by validated polymerase chain reaction (PCR) of the presence of *Brucella* genetic material in blood or other tissue sample(WHO., 2008).

• Demonstration by a validated serological method of *Brucella* antigen in blood or other tissue sample.

• Demonstration of a rising antibody titre in any serological test for brucellosis in the absence of exposure to any known source of cross-reacting antigens(WHO., 2008).

• Demonstration of a high sustained IgG antibody titre in the agglutination, complement fixation or ELISA tests with standardized antigens. (Ariza *et al.,* 2007)

Susceptibility to brucellosis in humans depends on various factors, including the immune status, routes of infection, size of the inoculum and, to some extent, the species of *Brucella.* In general, *B. melitensis* and *B. suis* are more virulent for humans than *B. abortus* and *B. canis,* although serious complications can occur with any species of *Brucella.* (Ariza *et al.,* 2007)

Common routes of infection include direct inoculation through cuts and abrasions in the skin, inoculation via the conjunctival sac of the eyes, inhalation of infectious aerosols, and ingestion of infectious unpasteurized milk or other dairy products. Blood transfusion, tissue transplantation and sexual transmission are possible but rare routes of infection. (Ariza *et al.,* 2007)

The disease is acute in about half the cases, with an incubation period of two to three weeks. In the other half, the onset is insidious, with signs and symptoms developing over a period of weeks to months from the infection. (WHO., 2008)

The clinical manifestations are varied and nonspecific. They include fever, sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain. Commonly, patients feel better in the morning, with symptomsworsening as the day progresses. The desire to rest can be profound, and depression is pervasive. If untreated, the pattern of the fever waxes and wanes over several days (“undulant fever”) (WHO., 2008)

Brucella species are facultative intracellular pathogens that can survive and multiply within phagocytic cells of the host. The mechanisms by which *Brucella* evades intracellular killing are incompletely understood. Nevertheless, *Brucella* organisms ultimately become sequestered within monocytes and macrophages of the reticuloendothelial system (RES), such as lymph nodes, liver, spleen and bone marrow. Brucellosis is a systemic infection that can involve any organ or tissue of the body. When clinical symptoms related to a specific organ predominate, the disease is termed “localized” . (WHO.,2008).

Although humoral antibodies appear to play some role in resistance to infection, the principal mechanism of recovery from brucellosis is cell-mediated. Cellular immunity involves the development of specific cytotoxic T lymphocytes and activation of macrophages, enhancing their bactericidal activity, through the release of cytokines (e.g. gamma interferon and tumour necrosis factor) from specifically committed helper T lymphocytes. Coincident with the

development of cell-mediated immunity, the host usually demonstrates dermal delayed type hypersensitivity to antigens of *Brucella.* (Banai *et al., 2007*)

Morphology:

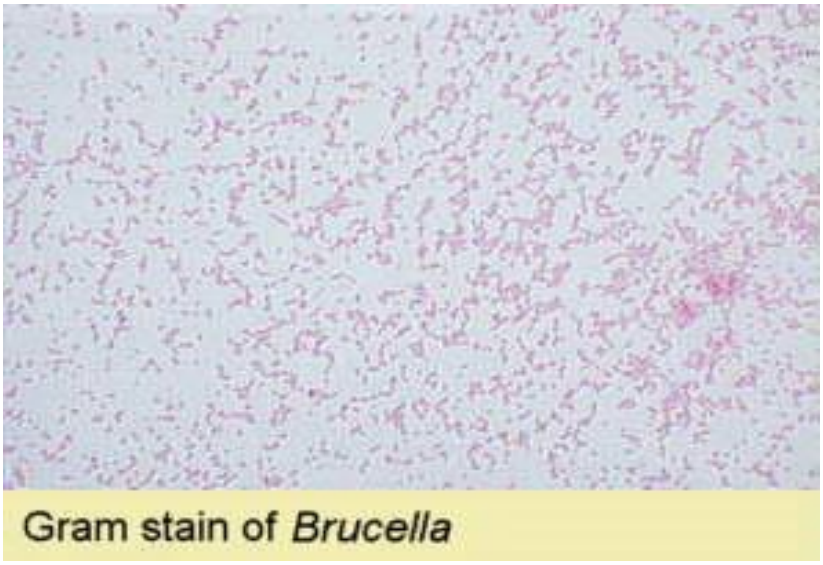

Figure 1.1 Gram negative coccobacilli of *Brucella spp*

Typically Brucella spp. occurs as small Gram-negative coccobacilli,

but coccal and bacillary forms may also occur. The cells are short and slender, the axis is straight; the ends are rounded; the sides may be parallel or convex outwards (Corbel, 1998).

In length they vary from about 0.6µm to 1.5 µm, and in breadth from 0.5 µm to 0.7 µm. The short forms may appear as oval cocci, or if they are arranged singly, in pairs end to end, or in small groups; sometimes short chains of 4-6 members may be seen, especially in liquid media. Because at their frequently coccoid appearance, their bacillary nature may be in doubt but it may be noted that they are smaller than any of the Gram-negative bacilli. Moreover, when arranged in pairs, their long diameter is in the same axis as that in which they are lying, as distinct from the gram-negative diplococci, whose long axis is generally at right angles to that in which they are lying. *B. melitensis* tends to be more coccal in form than *B. abortus* but this is not consistent enough to be of value for identification. The bacillary forms of *B. abortus* and *B. suis* are most readily apparent when grown on a rich medium, in which individual cells may reach 2-3 µm in length. *B. melitensis* usually remains cocoid and rarely exceeds I µm in length. The organisms stain fairly well with the ordinary dyes. They are Gram-negative, nonacid fast, non-motile and non-sporing. Bipolar staining can occur and

irregularity in the depth of colour may be seen especially in old cultures in which irregular forms appear (Corbel., 1998).

Brucella cells resist decolorization by dilute solutions of acids and alkalis, an advantage that has been utilized in a differential staining procedure known as Modified Z.N methods (Stamp *et al.,* 1950).

Classification:

The genus *Brucella* comprises a group of closely related species. Molecular genetic studies have indicated that the genus contains only a single species differentiated into a number of biovars, with certain host preferences (Corbel., 1998).

The taxonomic validity of this viewpoint has been accepted but the proposed new nomenclature, which would identify all members of the genus as biovars of *B. melitensis*, has been met with opposition on practical ground (Corbel.,1998). According to Jinkyung and Gary, (2003), currently there are seven nomen species classified as follows:

B. melitensis

Brucella abortus

Brucella suis

***Brucella ovis*:**

Brucella neotomae:

Brucella canis:

Brucella mans:

MERIALS AND METHODS

Study area and duration:

This study was carried out in Khartoum State, including blood donors in Central Blood Bank, during: March – May 2014.

Study population and sample size:

One hundred and fifty (150) samples were collected randomly from blood donors,in Central Blood Bank,in Khartoum State.

Ethical consideration:

Approval to conduct this study was obtained from the College of Graduate Studies, Sudan University of Science and Technology.

Permission was obtained from the blood donors , in Central Blood Bank, in Khartoum State, samples were taken from donors after their consent.

Specimens Collection:

Aliquots of five mls of whole venous blood were collected using sterile disposable syringes. And left to colot to 5 to 10 minetes The collected specimens were transported to the laboratory, refrigerated overnight, centrifuged for 3000 r.p.m for 5 minetes and serum was separated and stored at -20°C until tested.

Laboratory Methods:

Two serological techniques were used to detect anti-brucella antibodies in collected sera. The Rose Bengal Plate Test (RBPT) was used as screening test and the Standard Agglutination Test (SAT) as a confirmatory test.

Rose Bengal Plate Test:

Principle:

The test depends primarily on the reaction between the *Brucella* antigen and the specific antibodies that was assumed to be present in the sera of examined subjects.

The standardized buffered Rose Bengal stained antigen was kindly provided by the Veterinary Research institute (VRI), at Soba and was used to screen all the obtained sera.

Procedure:

Rose Bengal Plate Test (RBPT) was performed according to (Cheesbrough., 2000). The serum samples and antigen were brought to room temperature. 30Microliter of each serum sample were placed on a white plastic plate. After shaking the antigen bottle, an equal volume of the antigen was placed near each serum spot. They were mixed thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter. The mixtures were agitated gently for 4 minutes at an ambient temperature

on a rocker, after which the agglutination was read. Any visible agglutination was considered positive.

Standard Agglutination Test (SAT):

Principle:

This test was used to determine the antibody titer. It should also be performed when a patient with a negative test continues to show symptoms of brucellosis. The antigen reagent was kindly provided by VRI at Soba, Sudan.

Procedure:

Seven sterile small glass agglutination tubes labeled 1 to 7 were placed in a rack and 1 : 20 dilutions of serum was made in tube 1. One rnl from the first tube was taken and proceeded to make serial dilutions with 1 ml of 10% phenol saline up to the 6th tube, while the 7th tube was left to serve as a blank or negative control containing only phenol saline. Then one drop(50 microliter) of the antigen suspension (two antigen reagents (Omega-from Biosystem) one of the reagent was specific to *B. abortus* and the other was specific to *B. melitensis*) was added into each tube, mixed well and incubated at 37°C for 24 - 48 hours. Each sample was two folds serially diluted as follows: $\frac{1}{20}, \frac{1}{40}, \frac{1}{80}, \frac{1}{160}, \frac{1}{320}, \frac{1}{640}$ + control (normal saline + antigen reagents) was placed aside two reagents.

Quality control:

Positive and negative control sera were run in parallel with each performed batch. Duplicates of each tested serum were used to assure that the antigens used in the test were sensitive as well as specific (Cheesbrough., 2000).

Data analysis:

Data analysis was done, using the Statistical Package for Social Sciences (SPSS). Chi-square test was used to assess the difference between the various groups. Statistical significance was taken as $P(< 0.05)$.

RESULTS

Out of the blood samples collected from 150 donors , males were 133 (88.7%) , females were 17 (11.3%). Twenty three(15.3%) out samples of the 150 blood donors were positive for *Brucella* species.

Twenty (13.3%) samples were positive in males and 3(2.0%) in females donors.Table,1 and Fig,1.

As shown in table,2 student and employees had the higher incidenece of Brucellosis with percentages 43.5% and 39% respectively table,2

Table 3 revealed the prevalence of *Brucella* species found in the blood donors, 21 (70%) were *B.abortus* species and were 9(30%) *B.melitensis* table 3 and Fig 2

Seven samples showed antibodies of both *B.abortus* and *B.melitensis* all from males.

The distrubuation of *Brucella* species according to gender are showen table 4 and Fig 3. In males donors *B.abortus* were 20 (66.7%) and *B.melitensis* were 7 (23.3%) . in females donors 1 (3.3%) was *B.abortus* and 2 (6.7%) were *B.melitensis*

Table(1): The percentage of positive sample in male and female among the sex and total samples

Sex	**Frequency**	**Positive**	**Percentage(total)**	**Percentage among sex**
Male	133	20	13.3%	15%
female	17	3	2.0 %	17%
Total	150	23	15.3%	

Table(2): The percentage of ***Brucella*** isolates according to occupation of donors .

Occupation	**No. of isolates**	**Percentage**
Student	10	43.5%
Abattoir	2	8.7%
Employee	9	39%
Cooker	1	4.4%
Unemployed	1	4.4%

Table(3): The percentage of ***Brucella*** species among total brucella isolates

Brucella species	**Positive**	**Percentage %**
B. abortus	21	70%
B. melitensis	9	30%
Total of species	30	100%

Table(4): The percentage of ***Brucella*** species amonge male and female

Sex	**species**	***B. abortus***	***B.melitensis***
Male	27	20(66.7%)	7(23.3%)
Female	3	1(3.3%)	2(6.7%)
Total	30	21(70%)	9(30%)

Table (5) Titration Results for Brucellosis among blood donors

Code	1/20	1/40	1/80	1/160	1/320	1/640	Control
1		+(M)					
2			+(A)				
3				+(A)			
4			+(A)				
5				+(M)			
6			+(A)				
7				+(A)			
8			+(A)				
9				+(A)			
10			+(A)				
11				+(A)			
12				+(A)			
13			+(M)	+(A)			
14		+(M)	+(A)				
15		+(M)	+(A)				
16			+(A)				
17				+(A)			
18		+(M)		+(A)			
19			+(M)	+(A)			
20			+(M)		+(A)		
21			+(M)	+(A)			
22			+(A)				
23			+(A)				

A: *B.*abortus

M: *B.*melitensis

$\frac{1}{40}$ up to $\frac{1}{640}$ were considered positive for *Brucella* species.

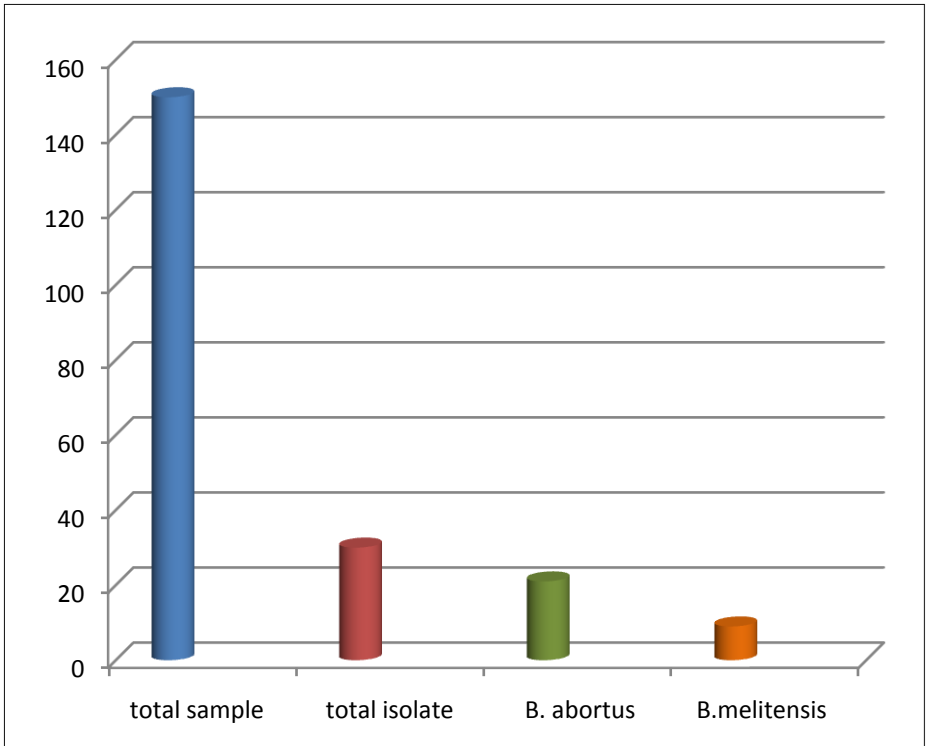

Fig 1: The percentage of ***Brucella*** species among total samples

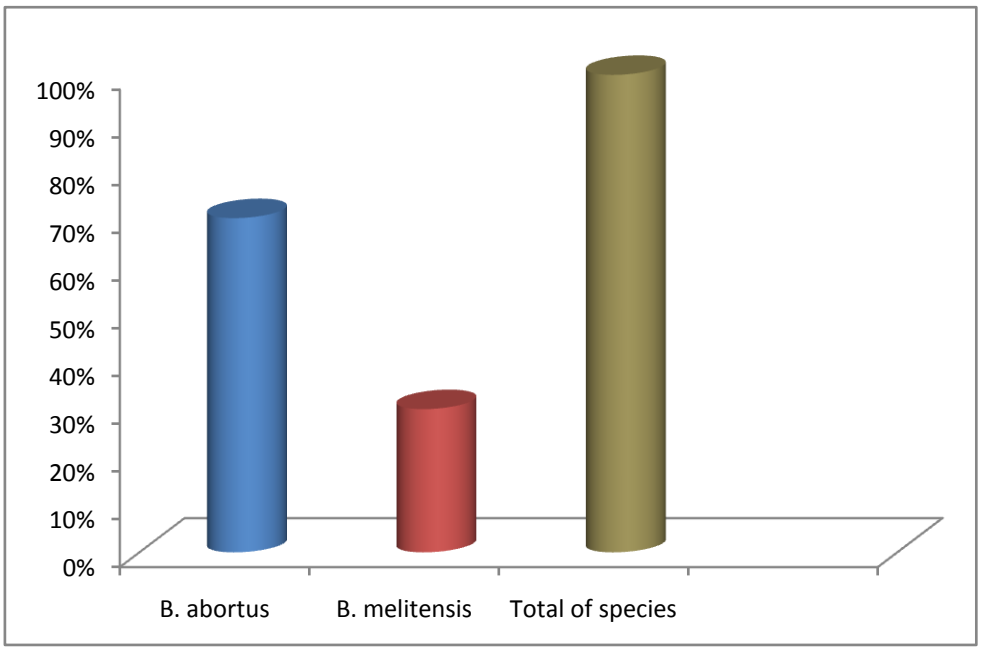

Fig 2: The percentage of ***Brucella*** species among the percentage of total ***Brucella*** isolates

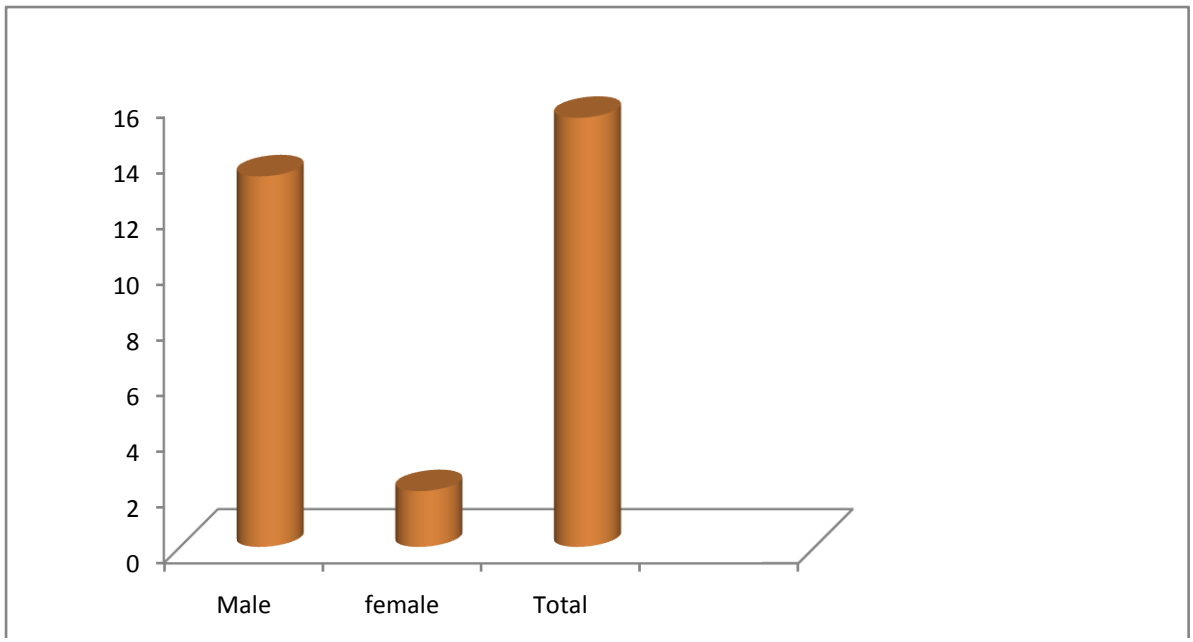

Fig 3: The percentage of positive sample in male and female among the percentage of total positive samples

DISCUSSION

Several studies were carried out on brucellosis in Sudan, but most of them were directed towards animal brucellosis Bennt.,(1943) , Dafalla and Khan ,(1958), Habiballa,*et al*., (1977), Sulima; (1987) , Musa *et al*., (1990) ,Suliman.,(2006). This study was attempt to understand the relation between blood donors and prevalence of brucellosis . The prevalence rate reported in this study was 15.3%, and it was higher than reported by (Mangalgi *et al*., (2012) in india and Bakhiet, (2004), Musa,(2004) who reported rate of 5.9% and 8% respectively in human in Sudan.

In my opinion, this difference might probably be due to the endemicity of *brucella* in Sudan . It is worthmenthioning that the occurrence of *brucella* in sudan is lower than the rate reported by Harding and Byers., (2000) . This high rate was attributed to the area of study where *brucella* is highly endemic.

students and employees had the higher incidenece of Brucellosis with percentages 43.5% and 39%. This may be due to bad hygeine mainly in type of food taken by these groups.

Out of the blood samples collected from 150 donors , males were 133 , females were 17 . Twenty (13.3%) samples were positive in males and 3(2.0%) in females donors. This may be due to the sample size .

In our opinion , there is risk of not including *Brucella* examination in the protocols of Sudanese blood banks. The risk is worsened by the fact that absence of symptoms even for along period does not necessarily ensure lack of infectivity and *Brucella* survive well in stored blood. Furtherly , this route of infection transmits brucellosis whereas most patients in need of blood transfusion are already weakened by severe disease. *Brucella* thus behaves very aggressively in such patients with higher risk of complication and fatalities .

This study showed significant probability value ($P<0.05$) among blood donors .

5.2 Conclusion

The high infection rate of *bruellosis* observed among the blood donors highlighted the risk of this pathogenic organism .

Prevalence of the disease among the blood donors are a potential and dangerous source to whom directly received the blood transfusion.

Recommendations

1. Screening of blood donors for *brucella* infection priors to donation.
2. Much knowledge about brucellosis has been accumulated, but not enough to master the disease. It is, therefore, necessary to acquire more knowledge.
3. To make this a reality, further in-depth studies are needed in human brucellosis by increasing the sample size of the people at risk and by employing the recent advanced molecular diagnostic techniques (e.g., PCR).
4. Because livestock animals and their products (meat, milk) are the major potential source for human infection, cooperation between veterinarians and human health care workers and epidemiologists is absolutely necessary to solve this problem once and for all.

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