



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



**Sudan University of Science and Technology**

**College of Veterinary Medicine**

**Serological Investigation of the disease**

**Brucellosis among Cattle in West Omdurman,**

**Khartoum State, Sudan**

**المسح المصلي لمرض الحمي المالطية للأبقار في السودان ،  
الخرطوم ، غرب أمدردمان**

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## Dedication

( وَقُلْ إِعْمَلُوا فَسَيَرَى اللّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ )

إلهي لا يطيب الليل الا بشكرك ولا يطيب النهار الا بطاعتك ولا تطيب  
اللحظات إلا بذكرك ولا تطيب الآخرة إلا بعفوك ولا تطيب اللحظات إلا  
برؤيتك الله جل جلاله ...

إلى من بلغ الرسالة وأدى الأمانة ونصح الأمة نبي الرحمة ونور العالمين  
سيدنا محمد عليه أفضل الصلاة وأتم التسليم

لملائكة الرحمة وعصفورات الجنة وغراسات المحبة اللاتي أرضعنا  
الحب والحنان أمهاتنا

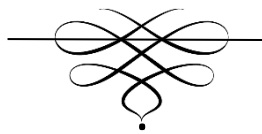
إلى اليد التي كلما سقطنا مدت لتضعنا على الطريق القويم العصا التي بها  
متكأنا آباؤنا

و أرواح آباؤنا التي تضيئ من على بعد عتمة الأفق المخيف

إلى من يقاسموننا حلو اللحظات ومرّها رفقاء دروبنا الذين يحتوننا دوماً  
اخوتنا وأصدقائنا

لمن بدأت معنا الخطى ولم تكملها فابتعدت وما ابتعدت آلاء حسن

و لكل من مر من هنا



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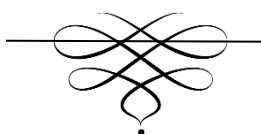
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# List of Contents

Contents:	page
الإهداء.....	I
Acknowledgements .....	II
List of contents.....	III
List of Tables.....	VI
List of Figures .....	VI
Abstract.....	VII
مستخلص الأطروحة .....	VIII
Introduction .....	1
<b>Chapter One</b>	
<b>Literature Review</b>	
1.1 Brucellosis.....	3
1.1.1 Definition.....	3
1.1.2 History of Brucellosis.....	4
1.2 Economic Importance.....	4
1.3 Geographic Distribution transmission.....	4
1.4 Transmission.....	5
1.5 Incubation period.....	5
1.6 Causes and pathogenesis.....	5
1.7 Clinical signs.....	6
1.8 Animal brucellosis.....	7
1.8.1 Bovine Brucellosis ( <i>B.abortus</i> ).....	7
1.8.2 Ovine and caprine Brucellosis ( <i>B.melitensis</i> ).....	8

1.8.3 Ovine epididymitis ( <i>B.ovis</i> ).....	9
1.8.4 Canine brucellosis.....	10
1.8.5 Horse Brucellosis .....	12
1.8.6 Brucellosis in marine mammals.....	14
1.9 Epidemiology.....	14
1.10 Diagnosis.....	14
1.10.1 Bacteriological methods.....	15
1.10.1.1 Microscopic examination.....	16
1.10.1.2 Culturing of samples for isolation.....	16
1.10.2 Guinea pig inoculation.....	16
1.10.3 Serological method.....	16
1.10.3.1 Rose Bengal plate test.....	17
1.10.3.2 Milk Ring Test (MRT).....	17
1.10.3.3 Serum Agglutination Test (SAT).....	18
1.10.3.4 Complement Fixation Test ( CFT ).....	18
1.10.3.5 Anti - globulin (Coomb's) test.....	18
1.10.3.6 Enzyme -Linked Immunosorbent Assay (ELISA).....	18
1.10.3.7 Indirect Haemagglutination Test (IHAT).....	19
1.10.3.8 Allergic Skin Test (AST).....	19
1.10.4 Molecular Methods.....	19
1.10.4.1 Polymerase Chain Reaction (PCR).....	20
1.11 Treatment of brucellosis.....	20
1.12 Control and prevention of brucellosis.....	20

## Chapter Two

### Materials And Methods

2.1 Study area.....	22
2.2 Husbandry and Management.....	22
2.2.1 Housing .....	22
2.2.2 Feeding.....	23
2.3 Sampling.....	23
2.3.1 Sources of samples.....	23
2.3.2 Collection of blood samples.....	23
2.4 Rose Bengal Test.....	23
2.5 Serum Agglutination Test.....	24

## Chapter Three

Results.....	25
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## Chapter Four

Discussion.....	33
Conclusion and Recommendations.....	34
References.....	35

## LIST OF THE TABLES

<b>Table</b>	<b>Page</b>
Table 1: The Result of Rose Bengal Plate Test of 70 examined cattle In West Omdurman - Sudan.....	28
Table 2: The Result of Serum Agglutination Tube for Brucellosis in 70 examined cattle In West Omdurman - Sudan.....	30

## LIST OF THE FIGURES

<b>Figure</b>	<b>Page</b>
Figure 1: The percentage of male and female among 70 examined cattle In West Omdurman –Sudan.....	26
Figure 2: The percentage of positive Cattle [n=70] to Brucellosis based on RBPT In West Omdurman –Sudan.....	26
Figure 3: The percentage of positive result of BBPT among females cattle In West Omdurman – Sudan.....	27
Figure 4: The percentage of positive Cattle [n=70] to Brucellosis based on SAT In West Omdurman –Sudan.....	27

## **Abstract**

The aim of this study was to monitor serological status of infections with brucellosis in some diary farms in west Omdurman, the total samples tested were 70 samples from adult cow ( 68 samples from females , 2 samples from males ).

All samples were tested by Rose Bengal and by Serum Agglutination tube test.

Rose Bengal Test revealed that 2% (2 out of 70) were positive to Brucellosis. Serum Agglutination Test revealed that also 2% (2 out of 70) were positive to Brucellosis. These were the same sample positive in Rose Bengal Test. The positive sample were among the females only.

It could be concluded that the cattle in west Omdurman are not free from Brucellosis and more investigation should be done.

### **Keywords:**

Cows , *Brucella* , Rose Bengal Plate Test , Serum Agglutination Tube Test, control and prevention.



## مستخلص الأطروحة

الهدف من هذه الدراسة هو عمل مسح مصلي للإصابات بمرض البروسيليا في بعض مزارع الالبان غرب أدرمان .

العدد الكلي للعينات المختبرة ٧٠ عينة من الأبقار البالغة ( ٦٨ عينة من الإناث ، ٢ عينة من الذكور ) كل العينات اختبرت بالروز بنغال الصفيحي وإختبار أنبوب تلازن الدم أوتراص الدم المصلي .

إن إختبار روز بنغال الصفيحي أعطى ٢% ( ٢ من ٧٠ ) عينة موجبة للبروسيليا ، إختبار تراص الدم المصلي أعطى أيضا ٢% ( ٢ من ٧٠ ) عينه موجبه للبروسيليا ، الحيوانات التي وجدت مصابة بهذا المرض هي إناث الأبقار .

وفي الختام أن المواشي في غرب أدرمان ليست خالية من مرض البروسيليا ، وينبغي أن تجرى دراسات فحصية أكبر في تلك المنطقة .

### كلمات المفتاح:

الأبقار، البروسيليا ، إختبار الروز بنغال الصفيحي ، وإختبار أنبوب تلازن الدم أوتراص الدم المصلي ، التحكم والوقاية.



## Introduction

Cattle are most common type of large domestic ungulates . They are a prominent modern member of subfamily Bovinae which are the most wide spread species of genus *Bos* . Cattle are raised as livestock for meat ( beef ) and dairy animals for milk and other dairy products , and as draft animals ( Bollongimo *et al.* , 2012 ).

Sudan cattle are two Principal varieties Baggara and Nilotic , The Baggara are two subvarieties constituted about 80% of the cattle, Nilotic cattle constitutes approximately 20% of cattle .Brucellosis has major effect in public health hazard is disease in both animal and human (AL Iraqi *et al.* , 2009).

Brucellosis is caused by gram-negative bacteria of genus *Brucella* which are, non motile , non spore-forming , rod-shaped ( coccobacilli ) bacteria. They function as facultative intracellular parasites, which belong to family alpha-2 proteobacteriaceae. The genus *Brucella* has been sub divided into 6 classical *Brucella* species , namely *Brucella abortus* (cattle and buffaloes ) *B.melitensis* (goat) ,*B.suis*(pigs,reindeer) ,*B.ovis*(sheep) ,*B.neotamae*(desert wood rats) and *B.canis*(dogs) ,based on strong affiliation to specific natural hosts . In addition to the classical *Brucella* spp.,the genus has recently been expanded to include marine isolates , which have been divided into 2 species , *Brucella ceti* and *brucella pinnipedialis* based on their preferential hosts , i.e. cetaceans and pinnipeds respectively .outbreaks of bovine brucellosis are associated with abortion during the last trimester of gestation ,production of weak newborn calves , and infertility in cow and bull (Malik *et al.*,2013).

Unpasteurized milk and dairy product transmitted Brucellosis to human. The disease is transmitted to the animals by the mean of licking aborted fetus or ingestion of contaminated milk or colostrum's. The disease affects the economy by delay estrus cycle increases calving interval, birth of weak calves, infectious abortion, infertility, reduction in milk production and finally lead to subsequent culling ( Adil *et al.* , 2014).

Clinical signs is one of the diagnostic methods for brucellosis but it is not valuable in case of heifer and males In spite of the laboratory diagnosis is one of accurate methods of diagnosis ( Malik *et al.* , 2013) .

Brucellosis is diagnosed by Rose Bengal test, buffer acidified plate antigen test and milk ring test which are rapid highly sensitive screening tests and complemented by complement fixation test . Recently the most confirmatory test is ELISA and Fluorescent polarization test (Gall and Nielsen, 2004).

### **Objectives:**

1. To investigate the presence of Brucellosis in cattle in West Omdurman(Al Thawra [ 103] ) farms by Rose Bengal Test.
2. To use Tube agglutination test for testing and confirmation of Brucellosis in West Omdurman.
3. To increase awareness of the disease among cattle owners.

# Chapter One

## Literature Review

### 1.1 Brucellosis

#### 1.1.1 definition:

Brucellosis are known as Undulate fever or Mediterranean fever or Malta fever. Brucella are small, Gram-negative, non-motile, non-spore-forming, rod-shaped (Coccobacilli) bacteria. They function as facultative intracellular parasites, causing chronic disease, which usually persists for life. Brucellosis is a bacterial disease caused by members of the genus Brucella, is an important zoonosis and a significant cause of reproductive losses in animals. Brucellosis is usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs. Abortions, placentitis, epididymitis and orchitis are the most common consequences, although other syndromes are also reported. The main impact is economic loss and deaths are rare except in the fetus and neonate. Some Brucella species are also maintained in wildlife populations. Wildlife reservoirs including feral pigs, bison, elk and European hares. Complicate eradication efforts for *B. abortus* and *B. suis*. Marine mammal isolates of Brucella have recently been recognized in many species of pinnipeds and cetaceans, and there are concerns that these organisms might have a detrimental impact on some species . Most species of Brucella can infect animals other than their preferred hosts, when they come in close contact. Brucella species are human pathogens. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. Most cases are caused by occupational exposure to infection ingestion of unpasteurized dairy products. In the U.S., *B. suis* has been eliminated from commercial pigs and *B. abortus* has nearly been eradicated from domesticated ruminants. As a result, human brucellosis is rare. However, this disease remains common and serious problem in some parts of the world (OIE<sup>a</sup> , 2009).

### **1.1.2 History of Brucellosis :**

Brucellosis was first suspected to occur in humans presenting with symptoms such as malaise, anorexia, fever and profound muscular weakness. This was reported in 1861 and, as such, the condition was called "gastric remittent fever". The causative agent was isolated from the spleen of patients by a British scientist, Sir David Bruce who named it *Micrococcus melitensis*. The genus *Micrococcus* was derived from its morphology and the species name from "Melita", the Roman name for the Isle of Malta where the disease was first recognized based on the description of the clinical illness, Hughes changed the name from "gastric remittent fever" to "undulant fever" in 1897 (Gabriel, 2005).

### **1.2 Economic importance:**

Occurring of the disease worldwide in domestic and infect animals as well as humans creates a serious economic problem for the intensive and extensive livestock production system losses in animal production due to this disease can be of major importance primarily because of 20% decreased milk production in aborting cows. The common sequel of infertility increases the period between lactations. The average inter calving period of an infected herd prolonged by several months. In addition, it results in loss of calves and interference with the breeding program. This is of the greatest importance in beef herds, where the calves represent the sole source of income. Also there is high incidence of permanent infertility (Minda , 2014)

### **1.3 Geographical distribution:**

Brucellosis is a disease of worldwide distribution occurring in domestic as well as wild animals. It has been reported wherever animals are raised all over the world (Seifert, 1996). Although some of the industrialized countries in Europe and America have achieved eradication of brucellosis through intensive control and eradication schemes, the disease is still a serious problem in developing countries (OIE<sup>a</sup> , 2009).

## **1.4 Transmission :**

*Brucella spp.* is commonly transmitted to other animals by indirect or direct contact with infected animals or their discharges (OIE<sup>a</sup> , 2009).

Transmission in cattle occurs mainly by ingestion of contaminated feed and water by organisms, which are present in large numbers in aborted fetuses, fetal membranes and uterine discharge. However, infection through injured/intact skin, the mucosa at the respiratory system and conjunctiva frequently occur. Brucellosis is transmitted to human through contaminated and unpasteurized milk and milk products or by direct contact with infected animals or animal carcasses. Abortion materials, uterine exudates, and colostrum are highly infectious. Primary routes of infection include penetration of the oral or gastric mucosa through ingestion of unpasteurized or contaminated dairy products, inhalation and penetration of the ocular mucosa, or through direct inoculation into the bloodstream through abrasions in the skin or vaccination. Occupational exposure to animals or animal products is the most common risk factor for brucellosis. Abattoir workers, farm or dairy workers, veterinarians and veterinary assistants, as well as healthcare and laboratory workers are well-recognized risk groups (Gabriel, 2005).

## **1.5 Incubation period:**

The incubation period of brucellosis varies from a few days to several weeks. According to the prolong incubation period the disease could run an acute or chronic course. When Brucellosis is introduced into a clean herd of animals, it may run an acute course causing “abortion storm” in 50% or more of the pregnant animals. The incubation period is often tied up with the stage of pregnancy at the time of exposure(OIE<sup>a</sup> , 2009).

## **1.6 Cause and Pathogenesis:**

*Brucella* organisms are small, fastidious, non-motile, non-spore forming and facultative intracellular bacteria. They are either coccobacilli or short bacilli with a size range of 0.5-0.7/µm wide by 0.6-1.5/µm long . They can occur singly, in groups, or in chains, and grow well

on media containing blood or serum . Brucella organisms are Gram negative but often resist decolourisation following counterstaining. Biochemically, Brucella organisms oxidise certain amino acids such as L glutamic acid and L-asparagine and certain carbohydrates such as D-glucose and lerythritol. *Brucella abortus*, *B. melitensis*, *B. suis* and *B.neotomae* may occur as either smooth or rough strains expressing smooth lipopolysaccharide (S-LPS) or rough-lipopolysaccharide (R-LPS) as major surface antigens, while *B. ovis* and *B. canis* are naturally rough strain(Gabriel , 2005).

### **1.7Clinical sign :**

Incubation period of brucellosis is very variable and has been defined in several ways:

- 1\ As the period between exposure and abortion or
- 2\ The period between exposure and the first appearance of clinical disease or
- 3\ The period between exposure and before the first serological evidence of infection can be detected ( OIE<sup>g</sup> ,2009)

In cows that eventually abort, the length of the incubation period varies according to the time at which infection occurred. Cows infected at service abort after an average interval of 225 days where as those infected at seven months gestation abort around 50 days later. Among susceptible groups, abortion storms during the third trimester, retained placenta, and metritis are Pathognomonic. It has also been reported that about 20% are infected only once . The disease has been associated with infertility in Cattle, goats, sheep, dogs and pigs and abortion in cattle .Male animals develop orchitis, hygromas and Sometimes inflammation of the seminal vesicles(OIE<sup>a</sup> , 2009).

In humans, brucellosis has an acute, sub-acute or chronic course, and the incubation period is usually one to three weeks, however occasionally, it may be several months , irrespective of the course of the disease, the predominant signs are intermittent or



Irregular fever, backache, headache, anorexia, weight loss, weakness, mental depression and arthralgia. Joint pain is Common, with the sacroiliac joint being mostly affected during the acute stage. In the chronic stage, the knee joint is most often affected. Localized complications may occur and may involve the cardiovascular, gastrointestinal , genitourinary, hepatobiliary , osteoarticular, spleen, lymphatic's , pulmonary and nervous systems , resulting in various clinical signs . For example, involvement of the nervous system leads to neuro-brucellosis (Gabril , 2005).

## **1.8 Animal brucellosis :**

### **1.8.1 BOVINE BRUCELLOSIS :**

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and occasionally by *B. suis*. Infection is widespread globally. Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent (OIE<sup>®</sup> , 2009).

#### **Clinical Signs :**

The disease is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk. Diagnosis depends on the isolation of *Brucella* from abortion material, udder secretions or from tissues removed at post-mortem. Presumptive diagnosis can be made by assessing specific cell-mediated or serological responses to *Brucella* antigens. *Brucella abortus*, *B. melitensis* and *B. suis* are highly pathogenic for humans, and all infected tissues, cultures and potentially contaminated materials must be handled under appropriate containment conditions ( OIE<sup>®</sup> , 2009).

#### **Identification of the agent:-**

Presumptive evidence of *Brucella* is provided by modified acid-fast staining of organisms, *Brucella* found in abortion materials or vaginal discharge, especially if supported by serological tests. The

polymerase chain reaction methods provide additional means of detection. Whenever possible, *Brucella spp.* should be isolated using plain or selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes and male and female reproductive organs. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria. Polymerase chain reaction (PCR) can provide both a complementary and biotyping method based on specific genomic sequences ( OIE<sup>g</sup> , 2009 ).

### **1.8.2CAPRINE AND OVINE BRUCELLOSIS :**

*Brucella melitensis* (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis. Sporadic cases caused by *B. abortus* have been observed, but cases of natural infection are rare in sheep and goats. *Brucella melitensis* is endemic in the Mediterranean region, but infection is widespread world-wide. North America (except Mexico) is believed to be free from the agent, as are Northern and Central Europe, South-East Asia, Australia and New Zealand. Clinically, the disease is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk. Diagnosis depends on the isolation of *Brucella* from abortion material, udder secretions or from tissues removed at post-mortem. Presumptive diagnosis can be made by assessing specific cell-mediated or serological responses to *Brucella* antigens *Brucella melitensis* is highly pathogenic for humans, causing one of the most serious zoonoses in the world, and all infected tissues, cultures and potentially contaminated materials should be handled at containment level 3 ( OIE<sup>b</sup> , 2009 ) .

#### **Identification of the agent:**

Presumptive evidence of *Brucella* is provided by modified acid-fast staining of organisms, *Brucella* morphology in aborted material or vaginal discharge, especially if supported by serological tests. The polymerase chain reaction (PCR) methods provide additional means of detection. Whenever possible, *Brucella spp.* should be isolated using

selective or non-selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes, spleen, uterus, testes and epididymes. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria. Molecular methods have been developed that could also be used for complementary identification method based on specific genomic sequences ( OIE<sup>b</sup> , 2009).

### **1.8.3 OVINE EPIDIDYMITIS (*Brucella ovis*):**

*Brucella ovis* causes a genital infection of ovine livestock manifested by epididymitis, infrequent abortions, and increased lamb mortality. Passive venereal transmission via the ewe appears to be a frequent route of infection, but ram-to-ram transmission is also common. Infected ewes may excrete *B. ovis* in vaginal discharges and milk and, accordingly, ewe-to-ram and lactating ewe-to-lamb transmission could also be determinant mechanisms of infection. Accordingly, the ewes are as relevant as rams in the epidemiology of infection, and control or eradication of *B. ovis* is feasible only if females are included in the corresponding programme the demonstration of the existence of genital lesions (unilateral or, occasionally, bilateral epididymitis) by palpating the testicles of rams may be indicative of the presence of this infection in a given flock. However, this clinical diagnosis is not sensitive enough because only about 50% of rams infected with *B. ovis* present epididymitis . Moreover, the clinical diagnosis is extremely nonspecific due to the existence of many other bacteria causing clinical epididymitis. The most frequently reported isolates causing epididymitis in rams include *Actinobacillus seminis*, *A.actinomycete mcomitans*, *Histophilus ovis*, *Haemophilus* spp., *Corynebacterium pseudotuberculosis ovis*, *B. melitensis* and *Chlamydophila abortus* (formerly *Chlamydia psittaci*). It must be emphasized that many palpable epididymal lesions in rams are sterile, trauma-induced spermatic granulomas. Although cattle, goats and deer have been proved susceptible to *B. ovis* in artificial transmission experiments, natural cases have been reported only in deer. To date, no human cases have been reported, and *B. ovis* is considered to be non-zoonotic. However, in areas where *B. melitensis*

infection co-exists with *B. ovis*, special care is required when handling samples, which should be transported to the laboratory in leak-proof containers ( OIE<sup>e</sup> , 2009) .

#### **1.8.4 Canine Brucellosis:**

##### **Etiology:**

In dogs, brucellosis is mainly caused by *Brucella canis*, a Gram-negative (CFSPH , 2009).

##### **Transmission: ,**

In dogs *B canis* primarily enters the body by ingestion and through the genital, oronasal and conjunctival mucosa, but transmission through broken skin may also be possible. Most cases are thought to be acquired by venereal transmission or by contact with the fetus and fetal membranes after abortions and stillbirths. Puppies can be infected in utero, and may remain persistently infected even if they appear normal. Nursing puppies can be infected from milk, but the importance of this route is controversial. Other potential sources of infection include blood transfusions and contaminated syringes. *B. canis* can be spread by fomites. In conditions of high humidity, low temperatures and no sunlight, *Brucella spp.* can remain viable for several months in water, aborted fetuses, feces, equipment and clothing. *Brucella* species can withstand drying, particularly when organic material is present, and can survive in dust and soil. Survival is longer when the temperature is low, particularly when it is below freezing . Humans usually become infected with members of the genus *Brucella* by dogs, especially animals that recently aborted or gave birth, and after exposure to large amounts of the organism in laboratories (e.g., contact with bacterial cultures). However, the source of the organism could not be determined in some cases (CFSP, 2009).

## **Clinical Signs:**

*B. canis* can cause abortions and stillbirths in pregnant dogs. Most abortions occur late, particularly during the seventh to the ninth week of gestation. Lymphadenitis is common in infected dogs. The retropharyngeal lymph nodes may enlarge after oral infection, and the superficial inguinal and external iliac nodes after vaginal infection. Generalized lymphadenitis is also common. Other symptoms that are occasionally reported include lethargy or fatigue, exercise intolerance, decreased appetite, weight loss and behavioral abnormalities (loss of alertness, poor performance of tasks); however, most affected dogs do not appear seriously ill. Occasionally, discospondylitis of the thoracic and/or lumbar vertebrae can cause stiffness, lameness or back pain. Chronic uveitis, endophthalmitis, poly granulomatous dermatitis, endocarditis, osteomyelitis related to hip prostheses, and meningoencephalitis/ low grade meningitis have also been reported. Fever is rare. Many infected dogs remain asymptomatic. Dogs with brucellosis may recover spontaneously, beginning a year after infection, but recovery is more common after two to three years, and some dogs remain chronically infected for years. Deaths are rare except in the fetus or newborn (CFSPH , 2009).

## **Diagnosis:**

The rapid slide agglutination test (RSAT) and the tube agglutination test (TAT) are often used to detect antibodies to *B. canis* in dogs. Other serological tests that have been used either clinically or in research include AGID, ELISA, an indirect fluorescent antibody (IFA) test, complement fixation, a lateral flow immune-chromatographic assay (LFIA) and counter-immunoelectrophoresis (CFSPH , 2009) .

## **Treatment:**

Some affected dogs have been treated successfully with long-term antibiotics. Treatment usually requires a combination of two different antibiotics, but Enrofloxacin alone appeared to be successful in

one trial. A few case reports have also documented the successful treatment of chronic or recurrent endophthalmitis caused by *B. canis*. No treatment is certain to eliminate *B. canis*. Even when this organism seems to have disappeared, it may persist in tissues such as lymph nodes, spleen, uterus and prostate. Recrudescence is possible, especially when an animal is stressed. For this reason, euthanasia of infected animals is often recommended in kennels, and this option should also be discussed when the disease is found in a pet. Neutering can be used as an additional control measure in treated animals, if they are intact. Periodic serological monitoring may be able to detect rising antibody titers during recrudescence (CFSPH , 2009).

### **1.8.5 BRUCELLOSIS IN THE HORSE**

#### **Aetiology and epidemiology:**

Bacteria of the genus *Brucella* are nonmotile, aerobic, intracellular Gram-negative cocci, cocco-bacilli or short rods. *Brucella* spp. are transmissible to a wide range of species, and among the domesticated animals, cattle, horse, sheep, goats and pigs are most commonly affected . Wild animal species are also occasionally infected (Mair and Diver , 1989) .

#### **Clinical signs:**

In horses that develop clinical signs of infection the organism usually localizes in bursae (causing septic bursitis), tendon sheaths (causing septic tenosynovitis) and joints (causing septic arthritis). Less commonly, cases of vertebral osteomyelitis, abortion and infertility in stallions have been recorded. The commonest clinical diseases associated with *Brucella spp.* infection in horses are septic supraspinatus bursitis (fistulous withers) and septic supra-atlantal bursitis (poll evil). fistulous withers or poll evil are reported in 85 cases, and identified *B. abortus* infection in 80%. Chronic draining sinuses occur in both conditions . *B. suis* has also been isolated from horses with septic bursitis ,aborted equine fetuses ,and the internal organs of a mare with no external signs of disease ( Mair and Diver , 1989).

## **Diagnosis:**

Serological testing is recommended in suspected cases. The card test that is widely used for screening of *B. abortus* in cattle has poor specificity. The plate agglutination test is considered to be more sensitive and specific a titre  $\geq 1:50$  is considered positive.

Occasionally, false positive results will be obtained, and there are reports of *B. abortus* isolation from seronegative horses. Other serological tests can be used, including tube agglutination, complement fixation, Coomb's antiglobulin, mercaptoethanol and agar gel diffusion tests. Although a rising titre will establish an acute infection, this might not be seen in long-standing cases; in these circumstances, a high titre in combination with appropriate clinical signs should be considered diagnostic. Radiography can be useful in fistulous withers and poll evil cases to assess the extent of bursal distension and associated osseous damage (Mair and Diver, 1989).

## **Treatment:**

Treatment of brucellosis in horses generally involves combination of systemic antimicrobials and local surgical drainage/debridement of infected tissue. Although *Brucella spp.* are generally sensitive to Tetracyclines, Chloramphenicol, Streptomycin and some Sulphonamides, there may be insufficient penetration into infected tissues to achieve resolution of the infection. In addition, polymicrobial infections are common. The successful treatment of 3 horses using Clofazimine has been reported. Administration of the *Brucella* strain 19 vaccine tracts with antiseptic solutions and Dimethylsulphoxide may be helpful. Radical surgical debridement of infected bursal tissue, with or without curettage of the dorsal spinous processes in the case of fistulous withers may be necessary in some animals the surgery may be performed in the standing horse or under general anesthesia (Mair and Diver, 1989).

### **1.8.6 Brucellosis in Marine Mammals:**

Since 1990 , *Brucella* strains have been isolated from a variety of marine mammals species, including seal,dolphins,whale,and other species (Ewalt *et al.* ,1994 ; Ross *et al.* , 1996 ; Foster *et al.* ,1996 ; Clauareau *et al.* , 1998 ; Wyatt, 1999 ) . These isolates have been classified as *B.ceti* and *B.pinnipedialis* and referring to cetaceans and seals, respectively (Foster *et al.* , 2007) .

### **1.9Epidemiology:**

#### **Prevalence of infection:**

The epidemiology of brucellosis is complex and it changes from time to time. Wide host range and resistance of *Brucella* to environment and host immune system facilitate its survival in the populations. Brucellosis is the most common zoonotic infection worldwide. It is endemic in the Mediterranean region, the Middle East, Latin America and parts of Asia and Africa, but the epidemiology is changing over the last decades due to socioeconomic changes, improved disease recognition and eradication programmes ( Minda , 2014) .

#### **1.10 Diagnosis:**

Brucellosis signs are non-pathognomonic in livestock, and definitive diagnosis depends on laboratory testing. Laboratory diagnosis includes indirect tests that can be applied to milk or blood, as well as direct tests (classical bacteriology and direct strategy depends on the prevailing epidemiological situation of brucellosis in susceptible culture animals (livestock and wildlife) within a country or region ( Minda , 2014).



### 1.10.1 Bacteriological isolation of *Brucella*:

*Brucella* isolation from foetal and placental cotyledon were performed according to Farrell method (1969). Approximately 1ml of foetal abomasal contents and placental cotyledon collected were rubbed on to *Brucella* medium base supplemented with 5% horse serum and onto Farrell's medium. Selective medium, which is prepared by the addition of *Brucella* selective supplement (Oxoid,SR0083A) (containing Polymyxin B(as SO<sub>4</sub>) = 2,500IU, Bacitracin = 12,500IU, cyclohexamide = 50.0mg, nalidixic acid = 2.5 mg, nyastatin =50,000 IU, vancomycin(as HCL) = 10.0 mg), 5% horse serum , 50% methanol and 50% dextrose on both *Brucella* medium base and tyryptic soy agar. Milk samples for isolation of *Brucella* are processed according to Alton process (Alton 1988). The milk samples are centrifuged at 3000 rpm for 10 minutes to obtain the sediment cream mixture which then is cultured on both basal media (*Brucella* medium base supplemented with 5% horse serum) and Farrell's medium (*Brucella* selective medium). The plates are incubated in presence of 10% CO<sub>2</sub> and in normal air condition at 37 °C for 2 weeks. Vaginal swabs are streaked on to solid media similar to milk and placental cotyledon samples. The inoculated plates from different clinical specimen are incubated at 37°C both in the absence and presence of 10% CO<sub>2</sub> for up to 2 weeks. After the incubation, the suspected colonies are examined for *Brucella spp* growth. *Brucella*-suspected colonies are characterized by their typical round, glistening, pinpoint and honey drop-like appearance , positive Gram stain, modified Ziehl-Nelsen stain, motility(at both 37c° and 20c°), oxidase, catalase, urease production while negative for methyl red,voges proskuaer test, acid production on media containing glucose, citrate utilization, indole test, and no growth on Mac Conkey agar and non-hemolytic blood agar ( Minda , 2014) .

### **1.10.1.1 Microscopic examination:**

*Brucella* organism may be demonstrated by staining method smear can be made from fetal membrane, fetal stomach, vaginal swab. The result should be positive or negative, two methods to describe:

1-Modified Ziehl-Neelsen method.

2-Modified Koster method (Gabriel, 2005).

### **1.10.1.2 Culturing of samples for isolation:**

*Brucella* most readily to be isolated in the period following an infected abortion or calving, but isolation can also be attempted using postmortem. *Brucella* can be excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media (Gabriel, 2005).

### **1.10.2 Guinea pig inoculation:**

Guinea pig inoculation method is used more than culture especially from 200 contaminated materials, injection are made intramuscular, guinea pig is killed from 4 to 5 weeks after inoculation and subjected to five tube agglutination test recovery of the organism from spleen of the guinea pig or positive serum agglutination test at 1/10 over taken as evidence of the infection (OIE<sup>n</sup>, 2009).

### **1.10.3 Serological test:**

Crucial for laboratory diagnosis of brucellosis since most of control and eradication programs rely on these methods. Inactivated whole bacteria or purified fraction lipopolysaccharide or membrane proteins are used as antigens for detecting antibodies generated by the host during the infection. Antibodies against smooth *Brucella* spp. (e.g. *B. abortus*, *B. melitensis*, and *B. suis*) cross react with antigen preparations from *B. abortus*, whereas antibodies against rough *Brucella* spp. (e.g. *B. ovis* and *B. canis*) cross react with antigen preparations from *B. ovis*. Although several serological methods are currently available, these tests can be classified as screening tests (e.g. buffered antigen

plate agglutination - BPAT), monitoring or epidemiological surveillance tests (e.g. milk ring test), and complementary or confirmatory tests (e.g. 2-mercaptoethanol, complement fixation, ELISAs, and fluorescence polarization assay) (Birney and Muccullough , 1976) .

#### **1.10.3.1 Rose Bengal Plate Test:**

The RBPT is a rapid agglutination test that is used as a screening test for the detection of antibodies to brucellosis in livestock, wildlife and humans. The antigen was used in the field as Field Rose Bengal Plate Test (FRBPT) and in the laboratory as Laboratory Rose Bengal Plate Test (LRBPT). Briefly, a 40-well Rose Bengal plate was used for the test. Using a disposable glass Pasteur pipette one drop (approximately 30  $\mu$ l) of serum was placed on each well of the plate. After warming the Rose Bengal antigen at room temperature for 30 minutes, one drop was drawn using a disposable glass Pasteur pipette and placed alongside the serum on the plate. The serum and antigen were mixed thoroughly using an applicator stick and the plate rocked gently to allow mixing. After few minutes, the plate was examined for agglutination in good light . Any degree of agglutination was taken as positive and absence of agglutinates was considered to be negative. The results were recorded and the plate washed with dry before being re-used (Gabriel , 2005) .

#### **1.10.3.2 Milk ring test (MRT):**

The test is used to detect infected animals on a herd basis or to monitor clean herds . Its sensitivity is low when compared to ELISA. For example, when sera used for ELISA and milk for MRT were obtained from the same female animals, the former technique revealed more positive animals than the latter one .

The test has shown several shortfalls and these relate to low sensitivity, attributed to the presence of mastitis, vaccination with S19, use of soured milk in the test, and marked changes of ambient temperatures (Bishop, *et al.*,1990).The test is not applicable in sheep and goats due to the high fat content of their milk (Shirma , 2005) .

#### **1.10.3.3 Serum agglutination test (SAT):**

Although this technique has been used widely as a routine screening of brucellosis for decades in several countries, it has been shown to have limitations. Such limitations include the failure to differentiate natural infections from the effects of vaccination, and failure to detect *Brucella* antibodies following abortion or during early incubation (Midia , 2014) .

#### **1.10.3.4 Complement Fixation Test (CFT):**

Due to its high sensitivity and specificity, this test is regarded as the definitive test for the serological detection of infected animals and humans. Complement Fixation Test results are rarely complicated by non-specific reactions and unlike the SAT, the titre does not wane as the disease become chronic (Shirma 2005) .

#### **1.10.3.5 Anti- globulin (Coomb's) Test:**

The antiglobulin (Coomb's) test detects antibodies of the IgG2 type and use to confirm SAT results. The Coomb's test, although laborious, is particularly important when the SAT is positive and CFT results are negative or conclusive. However Coomb's test results are indicative for infection only when it titres are at least two times than titres of the SAT. This test's main limitation, is not all infected cattle show this ratio. The 2-mercatoethanol and the revanol tests detect specific IgG (, and are usually used to differentiate between infected and vaccinated cattle ( Akif *et al.* , 2016) .

#### **1.10.3.6 Enzyme-Linked-Immunosorbent-Assay (ELISA):**

The advent of the ELISA technique has improved the sero diagnosis of brucellosis. The technique was found to be more sensitive than other serological tests. Among the ELISA methods, the Competitive ELISA (C-ELISA) was found to be more robust and easy to perform compared to others. The c-ELISA has several diagnostic merits and these include high sensitivity and specificity, ability to differentiate vaccinated animals from naturally infected ones, or those infected with a cross-reacting organisms. Additionally, the c-ELISA can be used on either serum or milk samples from different species (Shirma 2005).

### **1.10.3.7 Indirect Haemagglutination Test (IHAT):**

The test was found useful for the diagnosis of brucellosis in animal and man. It uses LPS of *B. abortus* or intracellular antigen and could be carried out as a tube or micro titre plate test (Corbel 2006). The IHAT is highly sensitive but its specificity was offset by difficulty of interpreting reactions produced at low dilution of sera (Akif *et al.*, 2016).

### **1.10.3.8 Brucellin allergic skin test:-**

The skin test is an allergic test that detects the specific cellular immune response induced by *Brucella spp.* infection. The injection of *Brucella* gene, a protein extract of a rough strain of *Brucella spp.* is followed by a local inflammatory response in a sensitized animal. This delayed type hypersensitivity reaction is measured by the increase in skin thickness at the site of inoculation. This test is highly efficient in discriminating between true brucellosis cases and false positive serological reactions. The skin test is highly specific but its weak sensitivity makes it a good test for herds but not for individual certification. It cannot discriminate between infection and vaccination.

In 1997 it made an assessment of the diagnostic value of the Brucellin allergic skin test (AST) in a brucellosis false positive serological reaction and reported that allergenic skin test is to be more specific than RBT and CFT. Therefore, this test could be used as a confirmatory test on cattle non-vaccinated against brucellosis. This test is prescribed as an alternative test (Minda, 2014).

### **1.10.4 Molecular techniques :**

Molecular biological techniques have the advantage of shortening the time required to identify the pathogens and they may detect organisms directly in clinical specimens. For diagnosis and epidemiological studies of brucellosis, techniques such as the Polymerase Chain Reaction (PCR), Restriction Endonuclease Analysis (REA) and Restriction Endonuclease and Hybridization have been used, and offer high degrees of sensitivity and specificity. However, these techniques are too expensive to be used widely, they are more and

appropriate for differential diagnosis rather than for establishing prevalence ( Gabriel , 2005).

#### **1.10 .4.1 Polymerase Chain Reaction (PCR):**

The technique is a very useful tool for the diagnosis of brucellosis because of its simplicity, high degree of sensitivity and specificity together with its speed, variety in sample handling and risk reduction for laboratory personnel. Serum sample should be used preferentially over whole blood for the molecular diagnosis of brucellosis . The test was used to diagnose brucellosis in goats and it was shown to be more sensitive than the RBPT and culture techniques. Recently 160 bovine milk samples examined using PCR. It was able to detect *Brucella* DNA from 20 milk samples (12.5%) ( Akif *et al.* , 2016) .

#### **1.11 Treatment :**

Brucellosis is one of the drug-neglected diseases and treatment of brucellosis in domestic animal is not indicated due to intracellular localization. *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage .Treatment failure and relapse rates are high and depend on the drug combination and patient compliance. The optimal treatment for brucellosis is a combination regimen using two antibiotics since monotherapies with single antibiotics have been associated with high relapse rates. The combination of doxycycline with streptomycin (DS) is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis(Shirma , 2005) .

#### **1.12 Control and Prevention:**

Control and prevention programmers based on various strategies have been successful in prevention of the spread between animals, monitoring of brucellosis-free herds and zones. Elimination of infected animals by test and slaughter, strict control of movement of infected and suspected animals, mass immunization to reduce infection rate, and supporting specific education and training programmers have

all received attention in countries. Control and eradication of the disease, however, is highly dependent on national strategies, priorities and policies. Although vaccination has some limitations, especially with live attenuated vaccines, extensive application has been adopted in several countries especially where the disease prevalence was high. The vaccine preparations currently used in the field are those containing smooth *B. melitensis* Rev.1; rough *B. abortus* strain RB51; smooth *B. suis* strain S2; rough *B. melitensis* strain Millan *B. abortus* strain S19. 1 vaccines have been used for the control of the disease in several countries such as South Africa, Israel, Cote d'Ivoire<sup>25</sup> and USA, Mexico and Chile with varying success. Rev.1 vaccine was used extensively in areas where *B. melitensis* infection as high, especially in the Mediterranean and Middle East countries (Bertu and Willson , 1995) .

## **Chapter two**

### **Materials and Methods**

#### **2.1 Study area:**

The study was conducted in the Cattle dairy farm in West Omdurman AL-Thawra 103 , it is located at West Omdurman where is situated between 15o 38` N and longitudinal 32o -26` E. The total area extends over approximately 21.000 square kilometer. The climate of Khartoum province is an arid type which is characterized by a wide range in daily and seasonal temperatures. During cool season between Decembers to February, the weather is cool and dry with minimum daily temperature of 24 c°. The season is characterized by low humidity. A hot dry weather prevails between March to October, a temperature of 45 c° may occur during the day. The maximum rainfall is during the period from mid-July to September, in this season there is an increase in relative humidity with maximum 68% in August. It is more convenient to divide the year into a cool dry season, hot dry season and hot wet season ([www.Khartoum.gov.sd](http://www.Khartoum.gov.sd)).

#### **2.2 Husbandry and management:**

##### **2.2.1 Housing:**

The yard of each barns was designed for at least 20 cows (8 dry cows and 12 milking cows) based on about 3-4 square meters per cow. The designed total area was 600 square meters (30 × 20) for each bran relatively, which was shaded. The shade area laid along the whole western lengths and 400 square meters (267 square meters for milking cows and 133 square meters for the dry cows).The roof made up from hay (traditional Sudanese roof).The margin was made from steel pole. The depth of margins was 50 cm and is situated along the outside of the yard so that feeding can be done without entering the yard. The length of feeding area was 15 meters long, therefore allowing at least space of 0.85 meters per cow. The watering more than the 20 liters barrel plastics



or porsaline fixed to the ground by steel frame and supplied by pipes which was connected to the water system.

### **2.2.2 Feeding :**

The fodder was cut and fed either green or dry to the animals in their yards and the concentrates were fed in concentrating portion during milking time to the milker cows . The dairy nutrient requirements of various classes of the dairy cattle. The dairy cows were fed on the forage produced in farms. The forage production was allowed feeding the cereal fodder (Sorgum–Abu70) and leguminous fodder (Lubia-Sudanese groundnut cake hay). The remaining nutrients were met by feeding a concentrate diet.

### **2.3 Sampling:**

#### **2.3.1 Source of blood sample:**

A total of 70 serum sample were collected from Cattle during the period from May to June 2017, the average animal age was between three to eight year, and body weight was between 250-350 kg.

#### **2.3.2 Collection of blood sample:**

Blood samples were collected from jaguar veins of animals by using sterile needles and syringe about 5ml of the blood was collected in plain tube without any anticoagulant. The blood samples were put on cool pox immediately and kept on it until transportation to the laboratory. Serum was separated by centrifugation ( at 3000 rounds for 10 min) . Serum were decanted into abandove tube and stored at – 20 c<sup>o</sup> degree until further *B* use. (Malik *et al.* , 2013) .

#### **2.4Rose Bengal plate test (RBPT):**

The RBPT is a rapid, slide-type agglutination assay performed with a stained *B. abortus* suspension at pH of 3.6-3.7 and plain serum (Minda 2014). In the laboratory Rose Bengal Plate briefly a12 wells were used for the test by using micropipette 50µl of serum was placed on each well of the plate. After warmed the Rose Bengal antigen at room temperate for 10 minutes, and 50µl of antigen was added by using micropipette placed alongside the serum on the plate. The serum and antigen were mixed manually and the plate rocked gently to allow

mixing. After 4 minutes, the plate was examined for agglutination in good light. Then any degree of agglutination was taken as positive and absence of agglutination was considered to be negative. The results were recorded and the plate washed with water and allowed to dry before being re-used (Malik *et al.* , 2013).

## **2.5 Serum agglutination test (SAT):**

This test was the first developed serological test for diagnosis of Brucellosis, is based on bacterial antigen agglutination, particularly by IgM under neutral PH (Minda , 2014) .

The test was performed in test tubes of approximately 1–2 ml total volume by placing 0.8 ml of phenol saline (0.5% [w/v] phenol in 0.15 ml sodium chloride) into the first tube and 0.5 ml volumes of phenol saline in the remaining tubes of a series of six tubes. A volume of 0.2 ml serum was added to the first tube, mixed, and then 0.5 ml was transferred to the next tube. Further volumes of 0.5 ml were transferred to subsequent tubes and discharged from last tube to give a series of doubling dilutions. Equal volume of antigen was added to each tube (0.5 ml) and the tubes were incubated at 37 °C for 16-18 hours.

The tests was read against opacity standards prepared by diluting the working strength antigen by phenol saline (by mixing ,25 ml of p/s+,75 ml Ag-50 ml p/s+50 ml Ag-,75 ml p/s +,25 ml Ag)to correspond to 25%, 50% and 75% agglutination. Phenol saline was used as the 100% control positive, and the undiluted working strength antigen as the 0% control or control negative. The results was scored as the degree of agglutination (+ = 25%, ++ = 50%, +++ = 75%, ++++ =100%) over the serum dilution. In each set of tube compered with control tubes(Corbel , 2006) .

## CHAPTER THREE

### RESULTS:

All the examined animals were 70 adult cattle (68 females +2 male) as is shown in Fig 1.

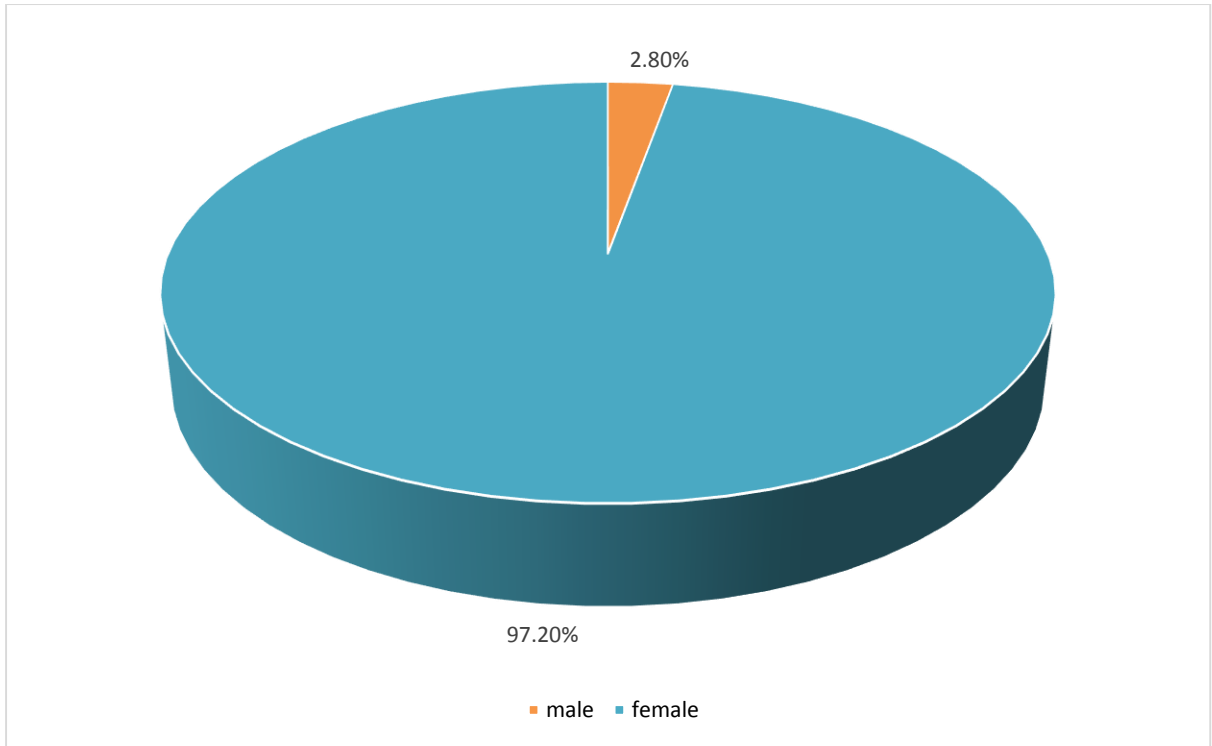
Fig 2 showed the percentage of positive and negative result among the examined cattle based on RBPT. The infection was low and constituted only 2.9%.

In Fig 3 showed the percentage of positive Rose Bengal Test among the examined females. The percentage of the positive females was 2.9%.

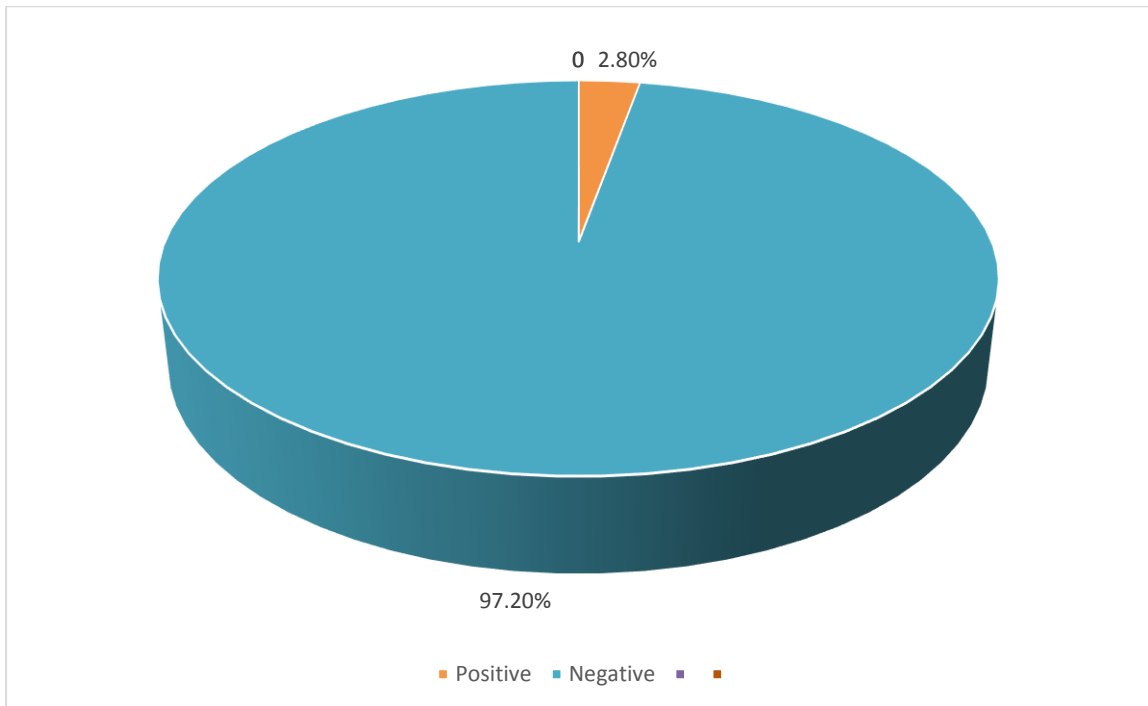
Fig 4 showed the percentage of positive and negative result among the examined cattle based on SAT. The infection was low and constituted only 2.9%.

As shown in table 1 the Rose Bengal Test showed that only two samples were positive to the *Brucella* antibody and were only among the females cattle.

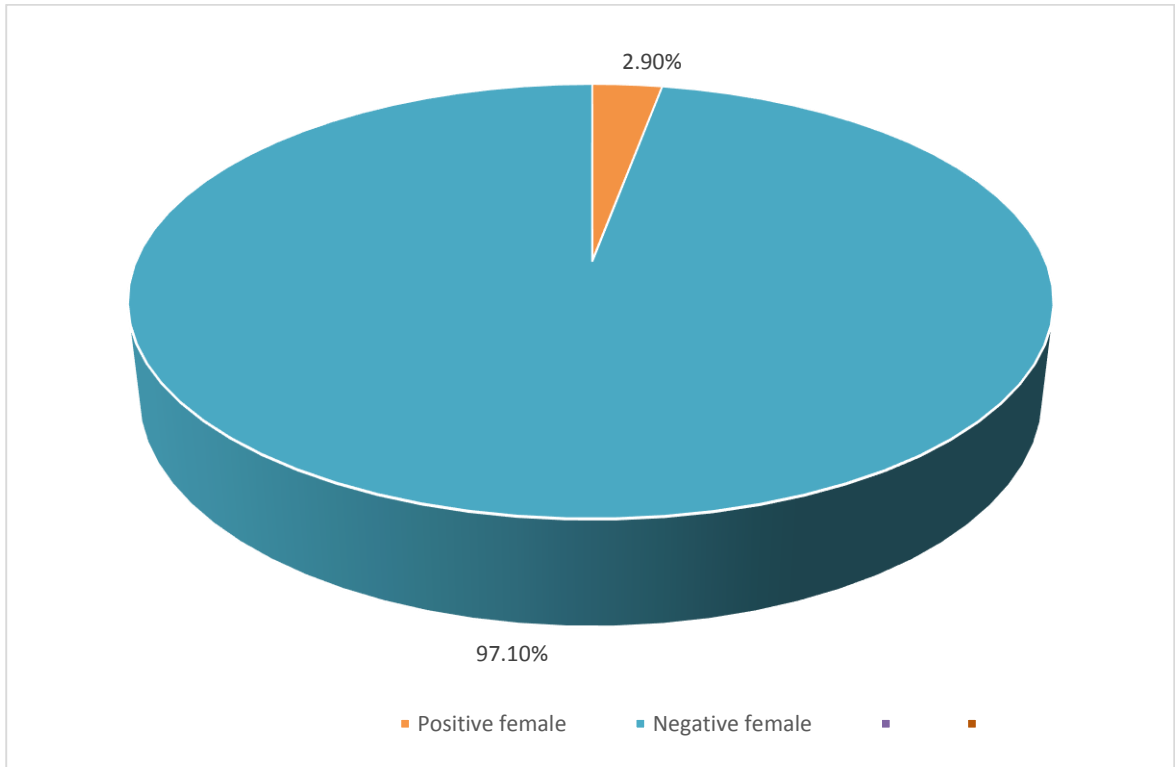
As shown in table (2) Serum Agglutination Tube test (SAT) revealed that the number of positive titer of antibody of *Bucella* was seen in only two cattle. The positive sample of SAT were the same as those found positive in RBPT.



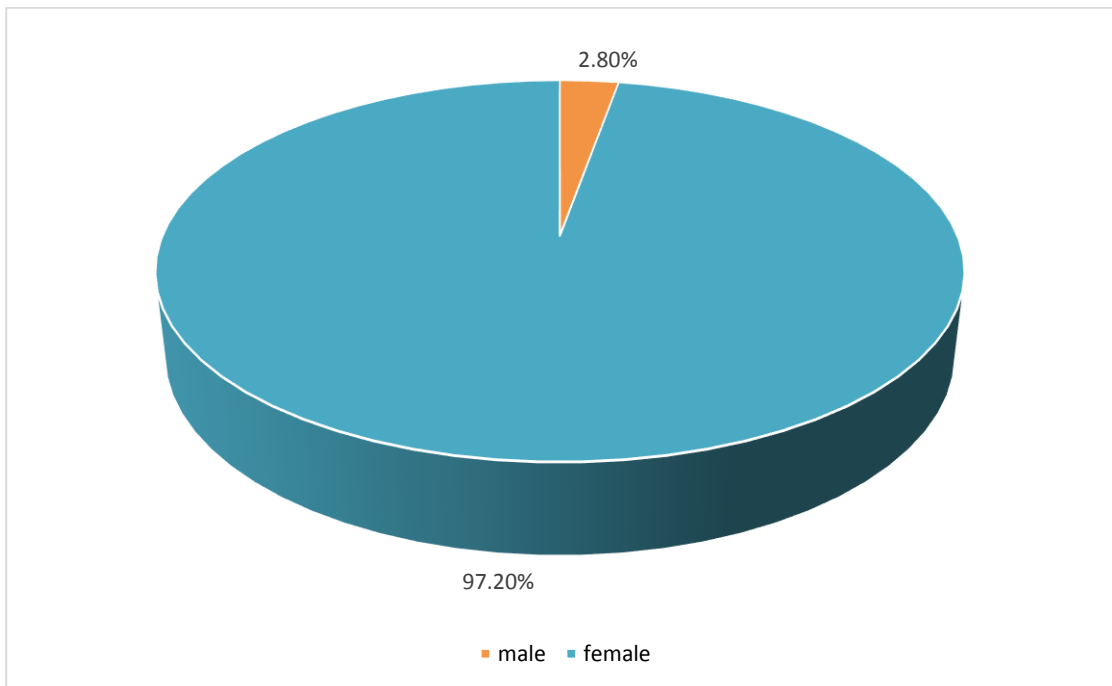
**Fig (1): The percentage of male and female among 70 examined cattle in west Omdurman - Sudan**



**Fig (2):The percentage of positive Cattle [n=70] to Beucellosis based on RBPT.**



**Fig (3): The percentage of positive result of RBPT among females cattle in west Omdurman – Sudan .**



**Fig (4):The percentage of positive Cattle [n=70] to Brucellosis based on SAT test in west Omdurman - Sudan .**

**Table 1: The Result of Rose Bengal plate test of 70 examined diary cattle in west Omdurman – Sudan :**

<b>Sample no</b>	<b>Cattle(sex)</b>	<b>Result Rose Bengal Plate Test</b>
1	Kamoon (male)	-ve
2	Karary (male)	-ve
3	Sarra (female)	-ve
4	Marwa (female)	-ve
5	Gadia (female)	-ve
6	Towma (female)	-ve
7	Marwan (female)	-ve
8	Gantera (female)	-ve
9	Dahab (female)	-ve
10	Gazal (female)	-ve
11	Cheeta (female)	-ve
12	Nagwa (female)	-ve
13	Amdignose (female)	-ve
14	Zamzum (female)	-ve
15	Biteenas (female)	-ve
16	Bitcheeta (female)	-ve
17	Sameera (female)	-ve
18	Nakhla (female)	-ve
19	Zytoon (female)	-ve
20	Mawada (female)	-ve
21	Akfa (female)	-ve
22	Alsamha (female)	-ve
23	Rawaa (female)	-ve
24	Omnia (female)	-ve
25	Hatim (female)	-ve
26	Aasul (female)	-ve
27	Rihab (female)	-ve
28	Wannasa (female)	-ve
29	Qamari (female)	-ve
30	Zarroqe (female)	-ve
31	Shama (female)	-ve
32	Samak (female)	-ve
33	Hawa (female)	-ve
35	Khartoumbellel (female)	-ve

**Table (1) .....Continued:**

36	Alrashid (female)	+ve
37	Ronka (female)	-ve
38	Bitbillia (female)	-ve
39	Bitruksha (female)	-ve
40	Sumra (female)	-ve
41	Bakhita (female)	-ve
42	Baladia (female)	-ve
43	458 (female)	-ve
44	459 (female)	-ve
45	462 (female)	-ve
46	Zihoor (female)	-ve
47	Warda (female)	-ve
48	Mashaer (female)	-ve
49	Reem (female)	+ve
50	Votka (female)	-ve
51	Annoff (female)	-ve
52	Ragda (female)	-ve
53	Habiba (female)	-ve
54	Biraghilalia (female)	-ve
55	Nabila (female)	-ve
56	Bitsallwa (female)	-ve
57	Buffra (female)	-ve
58	Qiseera (female)	-ve
59	Gameela (female)	-ve
60	106 (female)	-ve
61	107 (female)	-ve
62	108 (female)	-ve
63	109 (female)	-ve
64	110 (female)	-ve
65	Albicr (female)	-ve
66	Nazla (female)	-ve
67	Aashqa (female)	-ve
68	Basamat (female)	-ve
69	Zuhal (female)	-ve
70	Kowkab (female)	-ve

**Table 2: Result of Serum Agglutination Tube Test for Brucellosis in 70 examined cattle in west Omdurman - Sudan:**

Sample on	Cow (Sex)	Degree of Antibody
1	Kamoon (male)	-ve
2	Karary (male)	-ve
3	Sarra (female)	-ve
4	Marwa (female)	-ve
5	Gadia (female)	-ve
6	Towma (female)	-ve
7	Marwan (female)	-ve
8	Gantera (female)	-ve
9	Dahab (female)	-ve
10	Gazal (female)	-ve
11	Cheeta (female)	-ve
12	Nagwa (female)	-ve
13	Amdignose (female)	-ve
14	Zamzum (female)	-ve
15	Biteenas (female)	-ve
16	Bitcheeta (female)	-ve
17	Sameera (female)	-ve
18	Nakhla (female)	-ve
19	Zytoon (female)	-ve
20	Mawada (female)	-ve
21	Akfa (female)	-ve
22	Alsamha (female)	-ve
23	Rawaa (female)	-ve
24	Omnia (female)	-ve
25	Hatim (female)	-ve
26	Aasul (female)	-ve
27	Rihab (female)	-ve
28	Wannasa (female)	-ve
29	Qamari (female)	-ve
30	Zarroqe (female)	-ve
31	Shama (female)	-ve
32	Samak (female)	-ve
33	Hawa (female)	-ve
34	Khartoumbellel (female)	-ve



**Table (2) ..... Continued:**

35	Kamoon (female)	-ve
36	Alrashid (female)	+ + + + ve *
37	Ronka (female)	-ve
38	Bitbillia (female)	-ve
39	Bitruksha (female)	-ve
40	Sumra (female)	-ve
41	Bakhita (female)	-ve
42	Baladia (female)	-ve
43	458 (female)	-ve
44	459 (female)	-ve
45	462 (female)	-ve
46	Zihood (female)	-ve
47	Warda (female)	-ve
48	Mashaer (female)	-ve
49	Reem (female)	+ + + + ve *
50	Votka (female)	-ve
51	Annof (female)	-ve
52	Ragda (female)	-ve
53	Habiba (female)	-ve
54	Biraghilalia (female)	-ve
55	Nabila (female)	-ve
56	Bitsallwa (female)	-ve
57	Buffra (female)	-ve
58	Qiseera (female)	-ve
59	Gameela (female)	-ve
60	106 (female)	-ve
61	107 (female)	-ve
62	108 (female)	-ve
63	109 (female)	-ve
64	110 (female)	-ve
65	Albicr (female)	-ve
66	Nazla (female)	-ve
67	Aashqa (female)	-ve
68	Basamat (female)	-ve
69	Zuhal (female)	-ve
70	Kowkab (female)	-ve

\*Antibody Titer  $\geq$  50 I.U that is mean +ve.  
Antibody Titer  $\leq$  50 I.U that is mean -ve.

## Chapter Four

### DISCUSSION

In the present study, the occurrence of bovine brucellosis in west Omdurman using Rose Bengal Plate Test (RBPT) and Serum Agglutination test (SAT) . RBPT showed that 2.9% samples were positive ( 2 from 70 sample) and also 2.9% were positive (2 from 70 sample) when SAT was used .This finding is in disagreement with that reported at Sudan University of Science and Technology who recorded 100% negative from (25 sample) for RBPT( Akif *et al.* , 2016) . Also Hamid *et al.* (2014) found that the prevalence rate of brucellosis using RBPT in cattle in Bahari province 35.2% (207sample) . In Ibadan, Nigeria , Cadmus *et al.* (2006 ) reported that the prevalence of brucellosis in 1210 cattle was only 5.8% of samples were positive for RBPT .Angara *et al.* (2004 ) who recorded 93.3% of sample were positive for RBPT.

The present result although may be low (2.9%) but single every case should be considered a serious and more investigation should be done.

Since there was no history of Brucellosis in the examined area this may raise the worry of carrier animal to Brucellosis.

Also the absence of positive samples among the males may be due to small number of male examined.

## **Conclusion and Recommendation**

### **Conclusion :**

It could be concluded that the farm Al Thawra [ 103] which located at West Omdurman is not completely free from Bovine Brucellosis according to RBPT and SAT tests .

### **Recommendation:**

It is recommended that:

- 1- Extension program must be activated.
- 2- Control program should be planned to prevent spread of Brucellosis in animals and man.
- 3- Infected cattle must be isolated then culled and slaughter.
- 4- Healthy cattle must be vaccinated to prevent the incidence of disease in the study area.

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