Title:

Brucellosis in cattle in the Former West Kordofan State

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قال تعالى:
(والأنعام خلقها لكم فيها دفاء ونافع ومنها تأكلون)

صدق الله العظيم
سورة النحل، الآية (5)
Dedication

I would like to dedicate this work…

To…

all members of my family
My father and mother
My brothers and sisters.

To..

My wife Dr. Rasha.
And with love to my sons
Awab & Khabab.
Acknowledgements

Thanks first and last to Allah, who gave me the strength and patience to complete this work.

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**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verse from Holy Quran</td>
<td>I</td>
</tr>
<tr>
<td>Dedication</td>
<td>II</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>III</td>
</tr>
<tr>
<td>List of Content</td>
<td>IV</td>
</tr>
<tr>
<td>List of Table</td>
<td>VI</td>
</tr>
<tr>
<td>List of Figures</td>
<td>VII</td>
</tr>
<tr>
<td>Abstract</td>
<td>VIII</td>
</tr>
<tr>
<td>Abstract in Arabic</td>
<td>IX</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Economic importance</td>
<td>2</td>
</tr>
<tr>
<td><strong>CHAPTER ONE</strong></td>
<td></td>
</tr>
<tr>
<td><strong>LITERATURE REVIEW</strong></td>
<td></td>
</tr>
<tr>
<td><strong>1.1 Brucellosis</strong></td>
<td>4</td>
</tr>
<tr>
<td>1.1.1 Etiology:</td>
<td>4</td>
</tr>
<tr>
<td>1.1.2 Historical background</td>
<td>5</td>
</tr>
<tr>
<td>1.1.3 Taxonomy of the genus Brucella</td>
<td>6</td>
</tr>
<tr>
<td>1.1.4 Properties of Brucella</td>
<td>7</td>
</tr>
<tr>
<td>1.1.5 Molecular biology</td>
<td>9</td>
</tr>
<tr>
<td>1.1.6 Antigenic relatedness</td>
<td>9</td>
</tr>
<tr>
<td>1.1.7 Natural distribution</td>
<td>10</td>
</tr>
<tr>
<td>1.1.8 Transmission</td>
<td>10</td>
</tr>
<tr>
<td>1.1.9 Pathogenesis</td>
<td>11</td>
</tr>
<tr>
<td>1.1.10 Bovine brucellos</td>
<td>13</td>
</tr>
<tr>
<td>1.1.11 Human brucellos</td>
<td>14</td>
</tr>
<tr>
<td>1.1.12 The situation of brucellos worldwide</td>
<td>14</td>
</tr>
<tr>
<td>1.1.13 Brucellos in the Sudan</td>
<td>17</td>
</tr>
<tr>
<td>1.1.14 Diagnosis of Brucellos</td>
<td>18</td>
</tr>
<tr>
<td><strong>1.2 Methods of diagnosis include</strong></td>
<td>19</td>
</tr>
<tr>
<td>1.2.1 Bacteriological methods</td>
<td>19</td>
</tr>
<tr>
<td>1.2.2 Staining methods</td>
<td>19</td>
</tr>
<tr>
<td><strong>1.3 Isolation procedures</strong></td>
<td>20</td>
</tr>
<tr>
<td>1.3.1 Culture media of Brucella spp</td>
<td>20</td>
</tr>
<tr>
<td>1.3.2 Brucella growth characteristic.</td>
<td>20</td>
</tr>
<tr>
<td>1.3.3 Guinea pig inoculation</td>
<td>21</td>
</tr>
</tbody>
</table>
1.4 Serological methods: Different serological tests.

1.4.1 Rose Bengal Plate Test
1.4.2 Serum agglutination test (SAT):
1.4.3 Milk Ring Test (MRT)
1.4.4 Enzyme linked Immunosorbent Assay (ELISA)
1.4.5 Complement fixation test
1.4.6 Precipitation test
1.4.7 Whey agglutination test
1.4.8 Rivanol test
1.4.9 Indirect haemolysis test (IHT)

1.5 Molecular techniques: Polymerase Chain reaction (PCR)

1.6 Treatment

HAPETER TWO
MATERIALS AND METHODS

2.1 The study area
2.2 Livestock types
2.3 Animal diseases in the study area
2.4 Sterilization
2.4.1 Sterilization of equipments
2.4.2 Sterilization of culture media
2.4.3 Sterilization of solutions
2.4.4 Disinfection
2.5 Collection of samples
2.5.1 Blood samples
2.5.2 Milk samples
2.5.3 Hygroma aspirates
2.6 Serological tests
2.7 Tests procedures
2.7.1 Rose Bengal plate test:
2.7.2 Serum agglutination test
2.7.3 Milk ring test
2.7.4 ELISA test procedure
2.8 Staining techniques
2.8.1 Preparation of smears
2.8.2 Staining methods
2.8.2.1 Modified Ziehl Neelsen stain
2.9 The isolation of Brucella abortus from a knee hygroma in cattle
2.9.1 Primary isolation 43
2.9.2 Sub culturing of primary isolate 44
2.9.3 Purification of culture 44

2.10 Biochemical properties 44
  2.10.1 CO2 requirement 44
  2.10.2 Oxidase test 44
  2.10.3 Catalase test 45
  2.10.4 H2S Production 45
  2.10.5 Urease production 45

2.11 Biotyping of the organisms 45
  2.11.1 Growth in the presence of dyes 45
  2.11.2 Antigenic properties (agglutination with monospecific Brucella antiserum).

CHAPTER THREE
RESULTS
3.1 Results of serological tests 47
3.2 Prevalence of brucellosis in relation to herd structure 47
3.3 Bacteriological findings 47
  3.3.1 The isolation and identification of Brucella organisms 47
  3.3.2 Biotyping 48

CHAPTER FOUR
DISCUSSION
4.1 Seroprevalence of brucellosis 57
4.2 Brucellosis in relation to herd structure 58
4.3 Isolation of Brucella 58
4.4 Recommendation 59
References 60-67
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Table Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2-1)</td>
<td>Blood collected from cattle for serum samples from both sexes and different places</td>
<td>36</td>
</tr>
<tr>
<td>(2-2)</td>
<td>Blood for serum samples collected from different age groups of cattle</td>
<td>38</td>
</tr>
<tr>
<td>(3-1)</td>
<td>Results of the serological tests, RBPT, SAT, and ELISA in different localities</td>
<td>49</td>
</tr>
<tr>
<td>(3-2)</td>
<td>Rose Bengal Plate Test results of both male &amp; female cattle serum sample from different localities</td>
<td>51</td>
</tr>
<tr>
<td>(3-3)</td>
<td>RBPT results of different age groups of cattle from different localities</td>
<td>53</td>
</tr>
<tr>
<td>(3-4)</td>
<td>Milk ring test results</td>
<td>55</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Figure Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2-1)</td>
<td>Former West Kordofan and neighboring States and area of study</td>
<td>30</td>
</tr>
<tr>
<td>(2-2)</td>
<td>No. of blood samples for males and females in different localities</td>
<td>37</td>
</tr>
<tr>
<td>(2-3)</td>
<td>No. of blood samples collected from cattle of different age group in different localities</td>
<td>39</td>
</tr>
<tr>
<td>(3-1)</td>
<td>Results of the serological tests, RBPT, SAT, and ELISA in different localities</td>
<td>50</td>
</tr>
<tr>
<td>(3-2)</td>
<td>RBPT results for both male and female in different localities.</td>
<td>52</td>
</tr>
<tr>
<td>(3-3)</td>
<td>Prevalence of Brucellosis in cattle according to age group in FWKS</td>
<td>54</td>
</tr>
<tr>
<td>(3-4)</td>
<td>Milk ring test results</td>
<td>56</td>
</tr>
</tbody>
</table>
ABSTRACT

The objectives of this study were to determine the prevalence of brucellosis in the former West Kordofan State (FWKS) and to isolate and characterize Brucella species. Rose Bengal Plate Test (RBPT) and Milk Ring Test (MRT) were used as screening tests. Positive samples were confirmed by the Serum Agglutination Test (SAT) and ELISA. A total of 500 heads of cattle were examined. The prevalence rates were 24.6%, 23.6%, 23% and 5% by RBPT, SAT, ELISA and MRT, respectively. The prevalence of the disease was higher in females (34%) than in males (28.8%) and in cattle over three years old (47.5%). Hygroma was the most frequent clinical sign in cattle in the state. Hygroma aspirates from knee joints of 12 cattle in Lagawa locality Administrative Units (Asonot and Arak,) and serum samples from the same animals were examined by the RBPT, all samples were positive for Brucella antibodies. SAT was used to compare the level of antibodies in hygroma fluids and serum samples. The synovial fluids of knee Hygromas gave higher titers were a good source for isolation of Brucella organisms. Brucella abortus biovar3 strain was isolated from two samples of Hygroma fluid.
ملخص الطرح

هدفت هذه الدراسة إلى معرفة حجم انتشار المرض في ولاية غرب كردفان وعزل ووصف البروسيلا. استخدم اختبار الروز بنقال الصحي واللبين الحلقي كأختبارات تحديد المرض. واستخدم اختباري التراص المصلي والزيا كأختبارات تأكيدية للعينات الموجبة. فحصت 500 رأساً من الابقار في ولاية غرب كردفان وكانت نسبة الإصابة باختبار الروز بنقال الصحي 24.6% والتراص المصلي 23.6% والزيا 23% واللبين الحلقي 5%. وكانت النسبة أعلى في الإناث (34%) منها في الذكور (28.8%) وأيضاً كانت أعلى في الأعمار فوق الثلاث سنوات (47.5%). كان أكثر الاعراض انتشاراً التهاب المفاصل في ولاية غرب كردفان وفحصت 12 عينة من سوائل المفاصل الملتهب وعينات مصلية من الحيوانات نفسها بمحلية لقاح البروسيلا (الوحدات الدائمة السريعة والأراكن) باستخدام الروز بنقال وجميعها اعطت نتائج إيجابية. باستخدام اختبار التراص المصلي وجد ان كمية الأجسام المضادة للبروسيلا في سوائل المفاصل الملتهبة أعلى منها في الامصال. وعزلت عيارات من البروسيلا ابورتس بروفاز 3 من عينتين من سوائل المفاصل الملتهبة.
INTRODUCTION

Species of the genus *Brucella* cause diseases primarily in domestic, feral and some wild animals. However most are also pathogenic for humans. In animals, *Brucella* typically affects the reproductive organs and abortion is often the only sign of the disorder (Luhi, 1998).

*Brucella* are Gram-negative bacteria, small (0.5-0.7x0.6-1.5μm), non-motile, non capsulated coccobacilli, slow-growing and require complex media for isolation the genus *Brucella* comprises ten species *Br. abortus* which affects mainly cattle, *Br.ovis* affects sheep, *Br. canis* affects mainly dogs, *Br. neotomae* affects desert wood rat, *Br.melitensis* affects goats and sheep and *Br. suis* affects mainly pigs (Alshamahy , 1997). Humans are mainly infected with *Br. melitensis* but can be infected by *Br. abortus, Br. suis* and *Br. canis. Brucella* is often found carried by animals, but only incidental in humans’. It can inter the body via the mouth, vagina or wounds. Once there, this intracellular organism can enter the blood and the lymphatics where it multiplies inside phagocytes and eventually cause bacteremia (Bardstein et al., 2002).

In the Sudan, the *Brucella* was first isolated from a Friesian herd in Blue Nile Dairy Farm (Bennett, 1944). However the first isolation of *Br. abortus* from local cattle was from a cow which aborted at Juba Dairy Farm (ref). Prevalence of brucellosis in cattle in the Sudan was reported from different states. In Northern Sudan, Abdalla, (1964) reported a prevalence rate of 3% in Darfur states, Raga (2000) has reported a prevalence of 6.2%, El-Ansry et al., (2001) reported that 5% of cattle sera were found to be
positive in Kassala, Eastern Sudan. In Khartoum state, Khalid (2006) has reported a prevalence of 23.1%. However there was no report from. The former West Kordofan State.

**Economic importance:**

According to FAO/WHO expert committee on Brucellosis (1986), Brucellosis remains a major public health hazard and is an ever increasing cause of concern in many countries. The disease causes serious economic losses in terms of: Abortion, diminished milk production, culling of infected animals, rejection of consignment containing infected animals, costs on research control and eradication programmes, failure in financial investments, expenses on hospitalization, treatment of people and reduction of their working hours.

This investigation on cattle brucellosis was carried out in the former West Kordofan State, where the bulk of Sudan livestock is kept. Most cattle owned by nomads and raised in ranges. Cattle brucellosis is not studied in the state before; however there were some investigations conducted in the neighbouring states namely Darfur States, South Kordofan, North Kordofan and Unity states.

West Kordofan State used to stretch over a zone of semi arid to rich Savanna, between latitudes 9N-13 N and longitude 27 E-32 E in an area of 120,000 sq Km (28 million fedans). Natural grazing and forests represent about 85% of the area. It is one of the richest states in livestock (10\% of total cattle population in Sudan). Accordingly, animal resources are the fundamental source and support of livelihood. The total animal population in the state according to 2002 census was 9.16 million head as follows: cattle: 3.177.143, sheep:3.655.241, goats:1.917.696 and camels:411.265. The most prominent production system is the nomadic system, which depends mainly on continuous south/north movement on
traditionally known animals routes called (*Morhals*), searching for better pasture and water. The nomads of these *morhals* are Messyria. Baggara zebu is the main cattle type, characterized by tolerance of some disease and ability to travel for long distances. Crossing of local breeds with others like Foga, Kenana, and Botana Zebu cattle occurs in the towns. In spite of this large number of cattle, problems of animal health in the region received less attention from researchers, especially brucellosis.

Objectives of the study:

1- Determine the prevalence of brucellosis in the Previous West Kordofan State.

2- Compare the results of the different serological tests used for diagnosis of the disease.

3- Isolate and characterize of *Brucella* organism from cattle.

4- Recommend control measures accordingly.
CHAPTER ONE
LITERATURE REVIEW

1.1 Brucellosis:

Brucellosis is a contagious disease which primarily affects cattle, sheep, goats, swine and dogs. It is caused by the species of the genus *Brucella* and characterized by abortion in the females and, to a lesser extent, orchitis and infection of the accessory sex glands in the males, and infertility in both sexes. The disease is prevalent in most parts of the world. Brucellosis occasionally affects horses in which it is frequently associated with fistulous withers and poll evil. The human disease often referred to as undulant or Malta fever is a serious public health problem.

Most recently, *Brucella spp.* (different from those in land mammals) were isolated from dolphins, seals and an otter. Experimentally, at least one of the marine mammal species can infect and cause abortion in cattle, and marine mammal species have also been isolated from human beings.

1.1.1 Etiology:

Species of the genus *Brucella*:

The genus *Brucella* contains 10 species: *Br. melitensis, Br. abortus*, *Br. suis, Br. neotomae, Br. ovis* and *Br. canis*. *Br. melitensis* typically causes brucellosis of sheep and goats but also causes active disease in cattle and is the most important zoonosis in man. There are 3 biotypes of *Br.melitensis*, but they differ from one another serologically using monospecific A and M antisera.

*Br. abortu*, the cause of contagious abortion in cattle, has 7 biovars different from one another in biochemical reactions. All, however, are lysed by *Br. abortus* phages and show a characteristic
behavior in oxidative metabolic tests. Infection of animal species other than bovines with *Br. abortus* is common. Troublesome human infections occur quite frequently. *Br. suis* has 4 biovars, the first 3 being mainly pathogens of pigs though in the case of *Br. suis*, biovar 2, the European hare (*Lepus europaeus*), is involved in the epidemiology. Biotype 4 causes brucellosis in reindeer (*Rangifer tarandi*). *Br. suis* is highly pathogenic for man. *Br. neotomae* was isolated from the desert wood rat (*Neotoma lepida*), an animal inhabiting western regions of the USA. The importance of *Br. neotomae* as a pathogen is unknown as only 27 cultures have been isolated, none of them from domestic animals or man. *Br. ovis* causes widespread disease known as ram epididymitis, which is of great economic importance in most of the major sheep-raising areas of the world. Although the male is chiefly affected, transient infections in females also occur. *Br. ovis* is not known to cause disease in humans. *Br. canis* causes a highly infectious form of brucellosis in dogs, both sexes being affected. Infections in other species have not yet been reported, except for a few cases in man. Sporadic infections with *Br. abortus*, *Br. melitensis*, or *Br. suis* also occur in dogs (Alton, 1975).

*Brucella species* have also been isolated from several marine mammal species (pinnipeds and cetaceans).

### 1.1.2 Historical background:

The causative agent remained unknown until David Bruce (1881) isolated a microorganism from a spleen of a soldier with Malta fever and named it *Micrococcus melitensis*. Zammit, (1905) identified goats as the reservoir of brucellosis in Malta.

In man brucellosis is known as Malta fever, Mediterranean fever, remittent and goat fever, which are often synonymously used for undulant fever (Carpenter and Hubbert, 1963).
Human brucellosis is a systemic disease that has an acute or insidious onset. Symptoms include continuous or intermittent fever of variable durations, headache, anorexia, joint pains, a weakness, profuse night sweating and chills, malaise, lethargy, arthralgia, depression and generalized ache, and it is a flu-like disease. The manifestations of brucellosis are similar in neonates children and adults (Benenson, 1995).

In the Sudan the disease was diagnosed in humans as early as 1904 in a patient at Berber in northern Sudan (Haseeb, 1950), (Sympson, 1908) reported 20 clinically diagnosed cases in the Blue Nile and Kassala Provinces. Haseeb (1950) and Dafalla (1962) stated that the disease was diagnosed in all provinces except Bahr Elgazal in the south up to 1955.

The first isolation of *Brucella* organisms from animals was by Bang (1897). Who was the first to report contagious abortion in cattle and the other animal species and he named his isolate *Bacillus abortus*, which was followed by other names (*Corynebacterium abortus*, *Bacterium abortus* and *Alealigenes abortus*). Meyer and Shaw (1920) suggested the name *Brucella* for the genus. Prior to the use of the name brucellosis, the disease in animals was known by many names, such as infectious abortion, Bangs disease, slinking of the calf and contagious abortion.

1.1.3 Taxonomy of the genus *Brucella*:

Improvement of knowledge on the taxonomy of *Brucella* plays a significant role in solution of the questions of effective identification and differentiation study of museum cultures and their control as well as in scientifically grounded development of novel diagnostic and prophylactic preparations (Moreno, 1992). *Brucella*.spp are characterized by some definite enzymatic systems, e.g. .the presence of cytochrome C, C+G content, which is 56-58%, and homology index in DNA hybridization tests (over 90%). The common intracellular cytoplasmatic protein
fractions are regarded as a genus-specific criterion. It has been established that some definite structural components of O-polysaccharide chain of S-LPS stimulate cross-serologic reactions between the different species of *Brucella* and between *Brucella* and other related microorganisms (Ariza, 2003).

It has also been established that lipids extractions of microbial cells contain phospholipids and fatty acids typical only for *Brucella*. However, the problem of differential detection on species and biovar level within the genus still remains. The extending potencies of using modern methods of biochemistry, molecular biology and genetics in *Brucella* taxonomy will enable rapid and reliable identification of brucellosis agents (Kovach *et al.*, 1997). Several molecular genotyping methods have been used to show that *Brucella* species display significant, DNA polymorphism allowing the species to be differentiated, thereby justifying the current species classification (Corbel, 1973).

1.1.4 Properties of *Brucella*:

*Brucella* species survive well in aerosols and resist drying. The bacterium can survive in carcasses and organs up to 135 days, paper up to 32 days, soil up to 125 days and in blood at 4° C up to 180 days. They have a lipopolysaccharide coat that is much less pyrogenic than that of other Gram-negative organisms which accounts for the rare presence of high fever in brucellosis (Rodwan *et al.*, 1986)

Metabolism is aerobic; with oxygen acting as the terminal electron accepter via a cytochrome based electron transport system. Brucellae are positive for nitrate reductase, urease, oxidase and catalase, while they are methyl red, Vogues-proskauer and H2S negative. With the exception of *Br. neotomae*, none of the species forms acid from carbohydrates in peptone–water sugars. Carbon dioxide may be required for growth of
some strains. Growth occurs between 20-40°C, with an optimum growth temperature of 37°C. The optimum pH ranges from 6.6 to 7.4. Most strains require complex media containing several amino acids, such as thiamine and nicotinamide, magnesium ions, but X and V factors are not essential (Patrick et al., 2003).

*Brucella* is distinguishable from most other pathogens because it does not have obvious virulence factors like capsule, fimbriae, flagella, exotoxins, exoproteases or other exoenzymes, cytolysins, resistance forms, antigenic variation, plasmids, or lysogenic phages (Mareno and Mariyan, 2002). In addition to this, analysis of three *Brucella* have shown that their genomes lack the functional sequences of so-called classical virulence factors, pathogenic islands, and a complete set of genes to types 1, 2 and 3 secretion systems. It was found, however, that the bacterium recruits actins and activates guanosine triphosphate (GTPases) when internalized into cells (Mareno and Mariyan, 2002).

*Br. abortus* internalization and intracellular growth in non-phagocytic Vero cells has been shown by smooth virulent, smooth attenuated and rough strains of the bacterium replicated within the cells. Rough strains were more adherent and entered more Vero cells than the other strains. The smooth virulent *Br. abortus* strains replicated intracellularly at a larger percentage than smooth attenuated or rough strains. These differences in adhesiveness and invasiveness are due to hydrophobicity of the bacterium as measured by hydrocarbon adherence. Both intracellular smooth and rough brucella strains were found within cisternae of the rough endoplasmic reticulum. It was suggested that the movement to the rough endoplasmic reticulum is the limiting step in the infection of non-phagocytic cells. (Delleux et al., 1990).
1.1.5 Molecular biology:

All members of the genus *Brucella* produce similar patterns on basic electrophoresis of acid phenol soluble proteins (Fensterdank *et al.*, 1982). Based on the DNA-DNA hybridization studies, it has been shown that the genus *Brucella* is a highly homogeneous group and as a result, only one species *Br. melitensis* has been recognized in the genus. Ribosomal-ribonucleic acid DNA hybridization studies have indicated that the genus *Brucella* is related genetically to *Agrobacterium*, *Mycoplasma* and, *Phyllobacterium* and belongs to ribosomal ribonucleic acid super family (Araj and Azzam, 1996).

1.1.6 Antigenic relatedness:

The genus *Brucella* is a highly characterized by means of having the o-chain polysaccharides antigens which have recently been characterized at the molecular level in *Br. abortus* (Perry *et al.*, 1986). The structure (N-acylated-4-amino-6-dideoxy-D-mannose repeating units in o-chain) also exist with the O-chain of some other Gram negative bacteria which allow antibody cross-reactions. The known cross-reacting species or strains are *Yersinia enterocolitica* sero group O:9, *Salmonella* serotype N:30, *Escherichia coli* O:157 and O:116 serotypes, *Pseudomonas maltophilia*, *Francisella tularensis* and *Vibrio cholerae*. This potential for cross-reaction complicates the use of anti-LPS serum as a diagnostic agent unless the presence of other known cross-reaction species can be ruled out on other grounds (Nielsen and Duncan, 1990). However, DNA homology studies have shown that members of the genus Brucella lack homology with other micro-organisms having similar guanine+ cytosine percentage like *Serratia marcescens*, *Escherichia coli*, *Agrobacterium tumefaction* and the phenotypically
similar species. *Francisella tularensis* and *Bordetella bronchiseptica* (Ewalt et al., 2007).

1.1.7 Natural distribution:

Brucellosis was first recognized as a disease affecting humans on the island of Malta in the 19th and early 20th centuries. *Brucella* organisms can be found worldwide, but brucellosis is more common in countries having poorly standardized ineffective animal public health programs (Chamel, 1992)

Biovarieties vary with respect to geographic regions *Br. abortus*, *Br. ovis* and *Br. canis* are more widespread. *Br. melitensis* and *Br. suis* are irregularly distributed. *Br. neotomae* infection of humans or domestic animals has not been reported, and its distribution appears to be limited. Domestic and wild animal reservoirs may serve as sources of infection of livestock and humans (Spink, 1956).

1.1.8 Transmission:

Brucellosis is generally introduced into herds by infected animals, the organism localizes in the reproductive organs and / or udder. Infected animals may shed high numbers of bacteria in milk, aborted fetuses, vaginal discharges, placental membranes, and birth fluids. Susceptible animals can become infected via ingestion of pasture, feed or water contaminated with these excretions (Spink, 1956). Artificial insemination with infected semen can result in infection of the recipient cow. In swine, natural breeding is an important method of transmitting the disease. Horses generally acquire the infection through contact with infected cattle or swine (Young and Cordel, 1989).

Most human infections result from physical contact with infected animals or their excretions or consumption of contaminated or uncooked
animal products. Infection can occur via inhalation, ingestion, skin abrasions or mucus membranes. Veterinarians, farmers, slaughterhouse workers, meat inspectors, and laboratory technicians and animal handlers are at higher risk of exposure to *Brucella* organisms. Transmission can also occur via organ transplantation, sexual contact, breastfeeding, or transplacentally. Pus, blood, bone marrow, synovial fluid, cerebrospinal fluid, urine and genital exudates from infected humans may be infective (Bricker and Halling, 1994).

Cow may lick the material or the genital area of other cows or the organisms with contaminated food or water. Despite occasional exceptions; the general rule is that brucellosis is carried from one herd to another by an infected or exposed animal. This mode of transmission occurs when a herd owner buys replacement cattle that are infected or have been exposed to infection prior to purchase.

The disease may also be spread when wild animals or animals from an affected herd mingle with brucellosis-free herd (Allardetservent *et al.*, 1988).

**1.1.9 Pathogenesis:**

The incubation period of brucellosis in cattle, bison, and other animals is quite variable ranging from about 2 weeks to 1 year and even longer in certain instances; a typical incubation period would be 1-8 weeks (Gerald *et al.* 2004). *Brucella* species differ markedly in their capacity to cause invasive human disease. *Br.melitensis* is the most pathogenic and *Br. abortus* is associated with less frequent infection and a greater proportion of subclinical cases. The virulence of *Br. suis* strains for human varies but is generally intermediate (Allardetservent *et al.*, 1988).
The primary virulence factor for *Brucella* species is the cell wall lipopolysaccharide (LPS). Both smooth e.g. *Br. melitensis, Br. abortus, Br. suis* and rough forms e.g. *Br. canis*) exist with strains displaying rough LPS that have much less virulence in humans. After opsonization and ingestion by phagocytic cells, organism can be maintained intracellularly within phagosomes. This occurs because of adenine and guanine monophosphate production, which inhibits phagolysosome fusion, oxidative burst activity, and tumor necrosis factor production (Michelle et al. 2001). Animals studies suggest that invading brucellae are rapidly phagocytosed by polymorphonuclear leukocytes. *Brucella* are frequently able to survive and multiply in these cells because they inhibit the bactericidal myeloperoxidase-peroxide-halide system by releasing 5-guanosine and adenine. Early in infection, macrophages are also relatively ineffective in killing the intracellular *Brucella*. In systemic spread, it is not clear whether the bacteria are transported within neutrophils and macrophages or in the blood stream outside cells. Organisms may disseminate widely from regional lymphoid tissue and may localize in certain target organs such as lymph nodes, liver, bone marrow and (especially in animals) the reproductive organs. The presence of meso-erythritol in the testicles and seminal vesicles of bulls, rams and boars and in the products of conception in pregnant ruminants and pigs stimulates enormous multiplication of *Brucella*. Erythritol represents a potent localizing factor in the relevant species, but is absent in humans (Alton, 1988).

However, most of the mechanisms that allow the bacterium to cause tissue tropism as well as way in which *Brucella* enters and live in such different host cells such as epithelial cells are not known. *Brucella* is generally known for causing joint infections, abortion and other
reproductive complications in animals. The bacterium is also the cause of a rare, more severe systemic infection called neurobrucellosis, which results in the formation of *Brucella* granulomas in the central nervous system (Sohn *et al.*, 2003).

In humans, tissue lesions produced by *Brucella* spp consist of minute granulomas that are composed of epithelioid cells. In cases of infection with *Br. melitensis* these granulomas are particularly small, although the toxemia associated with this organism is great. Necrosis is not common, and abscesses do not form, except in *Br. suis* infection. The fact that humans rapidly develop hypersensitivity to *Brucella* antigens suggests that many of the symptoms of human brucellosis result from the reaction of the host defenses (Alton, 1988).

1.1.10 Bovine brucellosis:

In cattle, brucellosis is primarily a disease of the female, the cow. Bulls can be infected but they do not readily spread the disease. *Brucella* organisms localize in the testicles of the bull and produce an orchitis (inflammation of the testicles), whereas in the female the organisms localize in the udder, uterus, and lymph nodes adjacent to the uterus. The infected cows exhibit symptoms which may include abortion during the last third of pregnancy, retained afterbirth, and weak calves at birth. Infected cows usually abort only once. Subsequent calves may be born weak or healthy and normal. Some infected cows will not exhibit any clinical symptoms of the disease and give birth to normal calves.

*Brucella* organisms are shed by millions in the afterbirth and fluids associated with calving and aborting. The disease is spread when cattle ingest contaminated forages or lick calves or aborted fetuses from infected cattle. Outside the animal, afterbirth, or aborted calves, *Brucella* bacterium is easily killed by sunlight, high temperatures and drying.
However it is difficult to control while it is in the animals, (Young and Cordel, 1989).

**1.1.11 Human brucellosis:**

In humans, *Br. abortus* causes undulant fever, a disease characterized by intermittent fever, headaches, fatigue, joint and bone pains, psychotic disturbances and other symptoms. It is contracted through exposure to *Br. abortus* contaminated milk or through infected organs from infected animals. Livestock and slaughter industry workers and consumers of unpasteurized milk or its products are at the greatest risk of contacting undulant fever. Transmission occurs through contact with the tissues of infected animals at slaughter or ingestion of unpasteurized milk or milk products derived from infected cattle (Zygmunt et al., 1994).

**1.1.12 The situation of brucellosis worldwide**

Bovine brucellosis was recorded in 120 out of the 175 (68.8%) countries of the world (Nielsen and Ducan, 1990). Cattle are the most important source of infection with Br. abortus (Corbel, 1989). More recently, *Br. abortus* was reported by 101 countries, *Br. melitensis* in sheep and goats by 50 countries and *Br. suis* by 33 countries (WHO Working Group Meeting on Brucellosis Control and Research, 1992). Corbel (1989) reviewed the situation of brucellosis globally and stated that: (Bovine brucellosis has been absent from European countries like Denmark, Finland, Iceland, Norway and Sweden for at least two decades. It was also eradicated from Holland, Switzerland, Germany and most part of Belgium, Australia, Bulgaria, Czechoslovakia, Hungary, Rumania, and the former Yugoslavia. The disease was brought to low levels in Poland and USSR and the remaining European countries. *Br. melitensis* infection in sheep, goats and cattle in Europe is confined to the
Mediterranean region. France, Spain, Portugal, Italy, Albania, Greece, Malta and Turkey. It is rare in Cyprus, sporadic in Yugoslavia and was reintroduced in Switzerland and Germany from the neighboring countries. It is also prevalent in the former USSR. *Br. suis* in Europe is confined essentially to biovar 2 which sporadically occurring in wild hares, wild pigs and occasionally in porcine brucellosis. *Br. canis* infection in Europe was identified in Czechoslovakia and Germany.

In North America bovine brucellosis was eradicated from Canada, but the infection still exists in wild bison herds. The country is free from *Br. melitensis* and *Br. suis* of pigs but *Br. suis* biovar 4 is present in reindeer in the Arctic region. In USA bovine brucellosis was eradicated from most areas and reduced in some. In States like Florida and Texas the existence of large herds of cattle with a high turnover of animals makes the application of control measures very difficult. *Br. melitensis* was eradicated from native animals in USA and *Br. Suis* biovars 1 and 3 are quite uncommon. *Br. canis* is widely spread in the country. In wildlife in USA brucellosis occurs due to *Br. abortus*, *Br. suis* and *Br. canis*. In Mexico, bovine brucellosis is widespread and *Br. melitensis*, *Br. suis* and *Br. canis* exist in the appropriate hosts. Occasionally *Br. melitensis* and *Br. suis* are transmitted to cattle and subsequently to man.

In Asia, Japan is free from bovine brucellosis, but *Br. canis* was introduced in 1990. In Arabian Peninsula and Israel *Br. melitensis* exists. *Br. abortus* is also prevalent in Syria, Israel, Saudi Arabia, Iraq and Yemen. In Iran *Br. abortus* is prevalent in cattle and *Br. melitensis* in sheep and goats. In Mongolia, *Br. melitensis* was found to be a major problem in cattle, sheep, goats and yaks. In Afghanistan and Pakistan bovine brucellosis due to *Br. abortus* exists. In India *Br. abortus*, *Br. melitensis*, *Br. suis* and *Br. canis* were reported occurring variably in
different states in the different animals. In Nepal Br. abortus, Br. melitensis and Br. suis exist. In Sri-Lanka and Bangladesh bovine brucellosis due to Br. abortus is prevalent. In the latter Br. melitensis also exists in sheep. In China Br. melitensis occurs in sheep and goats, Br. abortus in cattle and yaks and Br. suis biovar 3 in pigs. In North Korea bovine brucellosis was eradicated and other species are not prevalent. In Taiwan Br. canis and Br. abortus were reported and in Burma Br. abortus and in Thailand both Br. abortus and Br. suis. In Malaysia bovine brucellosis is sporadic and Br. suis is prevalent. The situation in Kampuchea, Laos and Vietnam is unknown. In Indonesia, bovine brucellosis caused by Br. abortus and porcine brucellosis caused by Br. suis biovar 1 and 3 are widely spread. In Philippines, Br. abortus, Br. canis and Br. suis are prevalent. Bovine brucellosis is eradicated from Australia and only Br. suis exists in wild pigs. In New Zealand Br. abortus exists but the disease is under eradication. In Pacific Islands both Br. abortus and Br. suis biovars 1 and 3 exist.

In Africa Br. abortus and Br. suis were reported in cattle. In Libya Br. melitensis was found in sheep and goats. The same species is a major problem in Tansisia. In Algeria and Morocco bovine, ovine and caprine brucellosis is reported. Both Br. abortus and Br. melitensis were reported in Mali, Mauritanian, Niger and Sudan. Bovine brucellosis caused by Br. abortus has been recognized as a major problem in Senegal, Ivory coast, Togo and Nigeria and Br. melitensis was reported in Cameroon, Senegal, Togo and Nigeria and Br. suis in Ivory coast, Senegal and Togo. In Chad Br. abortus and Br. melitensis were reported in cattle and goats. In Central African Republic, Congo, Burundi, Rwanda and Zaire Br. abortus was reported in addition Br. melitensis is occurring in Zaire
In East Africa, bovines brucellosis is common in Uganda, Kenya, Ethiopia, Tanzania, and Somalia.

1.1.13 Brucellosis in the Sudan:

Brucellosis in the Sudan was first reported from human cases as early as 1908 (Haseeb, 1950). *Br. abortus* was first isolated from a dairy farm in Khartoum (Bennett 1943). While *Br. melitensis* was isolated from goat’s milk and from British residents in the Gezira area in 1953 (Daffalla and Khan, 1958). The organism was also isolated from camels in Butana area (Agab et al., 1995). Many investigators reported the disease from different parts of the Sudan (Daffalla, 1962, Shigidi and Razig, 1971, Musa and Mitchell, 1985). In Southern Sudan El nasri (1960) reported (14.6 - 18%) prevalence rates of the disease in cattle, 6, 6% in goats, and 3% in sheep. In the same area, Bauman (1983) studied brucellosis in Madi-Dink and Umbaroro cattle and reported 6.5% and 22.5% prevalence rates respectively, but did not find any positive cases in sheep, goats, and humans. Dafalla (1962) found the rate of the disease between 8.7 and 10.7% in cattle, 4.2 to 50% in sheep, 2.5 to 3% in goats in The Gazira in central Sudan, and 15% in cattle in South Kordofan. Mustafa and Hassan (1969) reported 5.7 and (8.7%) prevalence rate in cattle in Blue Nile. In North Kordofan Ibrahim and Habiballa (1975) found 14.2 and 66.7% prevalence rate in cattle.

Musa (1995) studied the situation of brucellosis in Western Sudan (Darfur states) in man, cattle, camel, sheep, goats and equines. He found the highest prevalence rate in animals kept in intensive farming systems than in those kept under nomadic conditions. The prevalence of the disease in cattle was found to be 13.9% followed by camels 7.76%, goats 5.98%, sheep 3.52%, horses 4.9%, and donkeys 3.57.
In Kassala State, Hatim (2004) reported the prevalence rates of brucellosis in 210 cattle, 356 camels, 2000 sheep and 2000 goats to be 11.9%, 19.1%, 0.3% and 0.45% respectively.

In greater Kordofan surveys were conducted in 2007 in both South and North Kordofan states funded by Western Sudan Resources Management Programme (WSRMP), and the findings were as follows: In South Kordofan state the prevalence rate of brucellosis in sheep was 95%, followed by goats 87.5%, cattle 85.9%, camels 83.3% and the lowest was detected in equines 6.7%. In North Kordofan state. In cattle, the highest prevalence rate of brucellosis among the localities was detected in Bara 40.5% followed by Sodari 33.3% and Anohud 30%. However, records of 25% and 21% were detected in Abu Zabad and Gubeish respectively; 16.2% for Sheikan and Um Ruwaba each, and 15.6% for Wad Banda. For sheep, Brucella prevalence ranged between a lower percentage of 13.9% in Um Ruwaba and higher percentage of 31% in Gubeish. In goats, values ranged between as low as 6.9 % in Gubeish locality to as high as 37.9% in Wad Banda. For camels the prevalence rate was highest in Abu Zabad (29.7%) followed by Gubeish (26.7%) and Bara ((19.4%), whereas it was relatively lower in Um Ruwaba (16.1%), Anuhud (14.3%) and Wad Banda (13.3%). However the prevalence rate in Sheikan (2.7%) was comparatively lower compared to Sodari (13%) and Jabrat (12.5%).

1.1.14 Diagnosis of Brucellosis:

Tentative diagnosis can be made clinically if there is history of exposure. Blood culture may be positive early in the disease course but serology is the mainstay of diagnosis. Interpretation is complicated by sub-clinical infections and persistent levels of antibody (Araj and Azzam, 1996)
1.2 Methods of diagnosis include:

1.2.1 Bacteriological methods

1.2.2 Staining methods.

1) Modified Ziehl –Neelsen method (Stamp et al., 1950).

2) Modified Kosters method. (Christofferson & Ottesen, 1941)

3) Immunofluorescence methods.

1) Modified Ziehl –Neelsen method (Stamp et al., 1950).

Brucella organisms are stained red against a blue background. In smears of fetal membranes they are often found in round clumps within tissue cells, which are stained blue. The organisms of enzootic abortion Chlamydia and Q fever are also stained red within tissue cells and are very difficult to differentiate from Brucella organisms. (Alton et al.; 1975)

2) Immunofluorescence methods.

An anti –Brucella serum conjugated with fluorescein isothiocyanate (fluorescent antibody (FA)) may be used to stain Brucella organisms. The fluorescent antibody technique may have special place in the examination of fetal membranes and aborted fetus when gross contamination is present and to clarify diagnosis, e.g. to differentiate between Chlamydia and Brucella. It has advantage of giving a quick result (1 to 2 hours) but the usual culture techniques are preferred and should be used in every case, whether an FA test is carried out or not. (Alton et al., 1975)
1.3 Isolation procedures:

1.3.1 Culture media of Brucella spp:

Except for special purposes, solid media are preferred for the isolation and further propagation of *Brucella* organisms. Solid media facilitate recognition and isolation of *Brucella* colonies and discourage dissociation. For the culture of certain fluids, notably blood, liquid media permit the culture of larger volumes than can be conveniently dealt with on solid media. Liquid media maintained under controlled conditions of oxygen tension, pH, etc., are finding increasing acceptance for mass cultivation of Brucella cells and for production of antigens and vaccines.

Some strains of *Brucella* require serum in the medium for their growth, especially on first isolation. Serum –dextrose agar, serum –tryptose agar and serum –trypticase –soy agar are recommended as the best basal media. Tryptose agar and trypticase-soy agar are available commercially in dehydrated form; 5% of serum is added after reconstitution and sterilization.

*Brucella* agar is a basal medium for cultivation of *Brucella* organisms. With the addition of 5% horse blood, the medium is used in qualitative procedures for cultivation and isolation of non fastidious and fastidious microorganisms from a variety of clinical and non clinical specimens (Ewalt *et al.*, 2007). Primary culture may be carried out on e.g. Serum –glucose agar or chocolate agar.

1.3.2 Brucella growth characteristic.

*Brucella* colonies generally become visible after cultures have been incubated for 3 days, but plates should be routinely examined on the forth or fifth day, when the colonies are round with smooth margins and 2-4mm in diameter. Growth is somewhat delayed on selective media. A
thorough search should be made as it often happens that only one *Brucella* colony is present on the plate. When plates are held up towards a light source (preferably indirect sunlight) and viewed through a transparent medium, the colonies are translucent and of a pale honey colour. Later they become larger and browner but remain clear. When viewed from above, the colony is convex and pearly white, or it may be slightly coloured by the dye if grown in medium containing ethyl violient. Occasionally colonies have a typical shape owing to distortion by fat globules or fragments of tissue left on the medium. Cultures regarded as negative on the fourth day of incubation should be re-examined on the eighth to the tenth day and may be discarded if still negative. (Alton *et al.*, 1975)

1.3.3 *Guinea pig inoculation.*

This method is more successful than direct culture especially from contaminated material. Guinea pigs are injected intramuscularly and killed after 4-5 weeks of inoculation. Then their sera are tested by Serum Agglutination Test. Recovery of the organism from the spleen or positive SAT at 1/10 serum dilution or over are taken as evidence of infection (Brinely, 1967).

1.4 *Serological methods: Different serological tests:*

Serology remains the mainstay of laboratory diagnosis. However interpretation of serology results is faced with difficulties. The large number of techniques in use is evidence of the problems. The standard serum agglutination test(SAT) has been augmented by the modified coombs (antigloulin) technique and the use of 2-mercaptoethanol to separate the actions of specific IgG and IgM. These classical methods may, in time, be supplemented by enzyme immunoassay test, (ELISA)
designed to differentiate between specific IgM and IgG antibodies (Bricher and Halling, 1994).

Serological testing is a valuable adjunct to culture—and molecular-based methods for the laboratory diagnosis of brucellosis. Various serologic assays have been described. These tests detect antibodies present in serum, milk, vaginal mucus and seminal plasma. The serological diagnosis is considered unreliable when performed during the period of 2 to 3 weeks before and after abortion or calving (Al-shamahy, 1997)

Very sensitive tests are in use of screening, and definitive ones for confirmation of infection. Usually more than one type of test are used because there is no single test which is both sensitive and specific, has ability to discriminate between vaccinated and non vaccinated animals and could distinguish between antibodies due to infection from those of cross reactions.

Many serological tests have been developed for diagnosis of brucellosis using body fluids such as sera, hygroma fluids, milk, vaginal mucus, semen and bursa and muscle juices. These tests are: Rosé Bengal Plate test (RBPT), serum and tube agglutination tests (SAT and TAT), complement fixation test (CFT), card test, plate agglutination test, modified SAT, Coombs test, enzyme linked immunosorbent assay (ELISA), milk ring test (MRT), and whey agglutination test. (Douglas et al 1987)

1.4.1 Rose Bengal Plate Test:

This is a rapid and simple screening test useful for detecting early infection (Corbel 2002). The RBPT or buffered Brucella antigen (BBA) is a rapid slide agglutination procedure for the direct detection of
Brucella antibodies in human and animal sera. The bacterial suspension is reactive with both IgG and IgM antibodies being detected earlier (sub-clinical infection) and for longer periods during the disease (chronic stage) than the conventional agglutination test. The assay is performed by testing the buffered (pH 3.6) suspension of Br. abortus strain 19 colored with Rose Bengal against unknown sera. The presence or absence of visible agglutination indicates the presence or absence of antibodies in the sample tested.

In the serological diagnosis of brucellosis in humans the RBPT test appears to have its main value in epidemiological surveys to delineate potential risk of infection in various population groups (Rose and Roeke, 1956)

1.4.2 Serum agglutination test (SAT):

The serum agglutination test will detect non-specific antibodies as well as those that are specific for Br. abortus infection and vaccination. SAT detects both IgM and IgG antibodies, although cross-reaction to Francisella tularensis may occur (Ariza, 2003). Febrile serum is used in agglutination test and aid in the diagnosis of certain febrile disease such as brucellosis. The patients serum is tested directly for homologous antibodies by either a slide or tube agglutination test. These tests are qualitative and semi-qualitative. The rapid slide test is especially useful when large numbers of sera must be examined. The tube test should be used to confirm positive results obtained by the slide test (Moreno, 1992).

The limitations of SAT are recognized. Not all infected cattle show a diagnostically significant titer, which may be negative during the incubation stage. In animals exposed for the first time during pregnancy, it not unusual for agglutination to appear only several days to two weeks after abortion or parturition. Also during the later stages of pregnancy, a
small proportion of animals show a transitory reduction in titer associated with the physiological movement of immunoglobulin from circulation to the mammary gland. The test may be negative in chronic infections also.

Following vaccination with strain 19, agglutinins may persist longer than antibodies detected by other tests. Heterospecific agglutinins due to cross reactions with other organisms, may interfere in some cases (Fensterdank et al 1982)

1.4.3 Milk Ring Test (MRT):

This test is a satisfactory and inexpensive test used for surveillance of dairy herds for brucellosis (Rose and Roepke, 1957). An adaptation of the agglutination test uses hematoxylin stained whole cell antigen added to milk, if antibody is present in the milk, it will attach to milk fat globules via its Fc portion. These antibodies will agglutinate with antigen and as the fat globules rise in the milk, a purple band will appear at the top of the milk. If no antibodies are present, the band will remain buff-colored (Velasco et al, 1998)

The MRT is the most practical method for testing lactating infected dairy herds and for surveillance of brucellosis free herds. It is performed on samples obtained from milk that is in cans or in bulk, and if repeated at least three times a year on each herd will detect the vast majority of infected herds. In eradication programmes, herd that show a positive ring test should be examined by serological test to identify infected animals. Continual surveillance of brucellosis free herds is done by milk ring test. Samples collected for routine determination of butterfat can be tested periodically by the ring test. The original procedure has been modified to maintain uniform sensitivity of the test under various conditions, i.e., for the examination of samples of cream, preserved milk,
milk from bulk tanks, and milk from very large dairy herds (Ashamahy, 1997).

Vaccination of adult animals with *Br. abortus* vaccine strain 19 can produce positive reactions to the milk ring test. False positive, suspicious reactions may be obtained during the drying period, if colostrum is present in the sample or if mastitis is present in the herd (Kovach *et al.*, 1997).

**1.4.4 Enzyme linked Immunosorbent Assay (ELISA):**

This test has been useful during eradication programs after vaccination has ceased and is used for screening or as a supplemental test to the complement fixation test. The ELISA test has superior sensitivity and reliability, and detects true negative results (Douglas *et al* 1984). Serum samples producing low level positive antibody titers in SAT, CFT and RBPT did not react uniformly when tested by five different commercial ELIZA test kits (Dabdoob and Abdulla, 2000).

**1.4.5 Complement fixation test:**

This test has good specificity and is the most reliable definitive test at the point in time aside from bacterial isolation (Letesson *et al* 1997). The complement fixation test can detect the presence of specific antibody in a patient serum. It uses sheep red blood cells (sRBC), anti-sRBC antibody and complement, plus specific antigen. If the antibody is present in the patient serum, then the complement is completely utilized, so the sRBCs are not lysed. But if the antibody is not present, then the complement is not used up, so RBCs are lysed (Crit, 1990).

**1.4.6 Precipitation test:**

Agar gel immunodiffusion (AGID) and single radial immunodiffusion (SRID) tests, were the first tests to distinguish vaccinal
antibodies from those resulting from field strain infection with *Br. abortus*. An antigen, polysaccharide B, derived from *Br. melitensis* was used, either incorporated into the agar matrix in SRID, followed by addition of the test serum to a well cut in the agar matrix or in the AGID, added to a well in the agar matrix adjacent to another containing the test serum. If antibody is present, a ring of precipitation would appear within a couple of hours or after a longer incubation period with sera containing fewer antibodies (Nielsen, 2002).

1.4.7 Whey agglutination test:

It is a tube test performed with whey milk. It is used when dealing with problem herds because it was claimed to be less affected by non-specific factors, yet it is less sensitive than MRT (McEwen, 1940).

1.4.8 Rivanol test:

Rivanol, 2-ethoxy-6, 9-diaminoacridine lactate, is added to serum causing high molecular weight glycoprotein to precipitate. The precipitate is then removed by centrifugation and a rapid agglutination test using serum diluted 1:25, 1:50, 1:100 is performed. This test is fairly labour intensive and mostly used as confirmatory test (Nicoletli, 1969).

1.4.9 Indirect haemolysis test (IHT):

An indirect haemolysis test (IHT) was compared with four other serologic procedures in sensitivity and specificity, using sera from bacteriologically confirmed *Brucella* infected herds. The test was more sensitive than the complement-fixation and tube agglutination tests, was similar to the Rivanol test, and was slightly inferior to the card test. It was more specific than the Rivanol and tube agglutination test but has less specificity than the complement fixation test. Results of all procedures were affected in varying degrees by the method of vaccination and time.
period of serum sample collection after vaccination (Dabdoob and Abdulla, 2000).

1.5 Molecular techniques: Polymerase Chain reaction (PCR):

PCR is a biochemistry and molecular biology technique for enzymatically replicating *Brucella* DNA. Like amplification using *Brucella* organism, the technique allows a small amount of *Brucella* DNA to be amplified exponentially. PCR is commonly used in medical and biological research laboratories for a variety of tests, such as the detection of *Brucella*. Serum samples should be used preferentially over whole blood for molecular diagnosis of brucellosis (Zerva et al., 2001). PCR was used to diagnose brucellosis in goats, cattle, and humans and it was shown to be more sensitive than the RBPT and culture techniques (Leal-Kievezas et al., 2000). It is used to diagnose brucellosis in goats, cattle, and humans and it was shown to be more sensitive than the RBPT and culture techniques (Leal-Kievezas et al., 2000). The development of PCR has offered a new dimension in the diagnosis of different microorganisms, which is possible in just a few hours. Over the past decade, there have been major advancements in all aspects of molecular diagnostic with regard to brucellosis. PCR-based tests are proving to be faster and more sensitive than traditional methods. However, the sensitivity and specificity of the PCR for *Brucella* vary between laboratories and no standardization of sample preparation, target genes, and detection methods have been established yet. All the important aspects of the PCR for *Brucella* DNA detection and its utility in diagnosis and follow-up of patients with brucellosis are reviewed (Letesson et al., 1997).
1.6 Treatment:

None of the currently available therapeutic agents, whether used alone or in combinations, were capable of killing all intracellular *Brucella* in vitro in 24 hours. A remarkable protection of intracellular *Brucella* against streptomycin has been demonstrated. The most effective reduction in the number of viable intracellular *Brucella* was accomplished by exposure of the host cells to streptomycin plus aureomycin, terramycin, or chloramphenicol.

The available evidence suggests that the ability of *Brucella* to localize and remain viable within the cells of an infected host is an important biologic factor in establishing and perpetuating *Brucella* infections, despite therapeutic measures or the operation of the host's humoral defense mechanisms. Reduction of neotetrazolium by leukocytes and *Brucella in vitro* provides a method for assessing the metabolic status of the host cell, but does not discriminate with any degree of certainty a viable from a non-viable intracellular organism.

A long term treatment with a high dose of oxytetracycline (1000mg/day/six weeks i/m), had completely eliminated *Brucella melitensis* from naturally infected sheep (Radwan *et al* 1986). In humans, however, adults and children older than 8 years, the use of doxycycline and rifampin for 4-6 weeks in the therapy of choice .Relapse rate is 5-10% in children younger than 8 years, the use of rifampin and trimethoprimsulfamethoxazole (TMP-SMX) for six weeks in the therapy of choice. Relapse rate appears to be approximately 5% or less (Velasco *et al* 1998).Doxycycline and rifampin form the basis of treatment .with contrimoxazole replacing doxycycline in children, but fewer relapses are reported with regimens including two weeks of daily streptomycin. Azithromycin has been shown to give promising results in experimental models Bricker and Halling, (1994).
CHAPTER TWO
MATERIALS AND METHODS

2.1 The study area:

Previous West Kordofan State was stated in 1994. It had an area of about 111,373 sq. km. Al-Fola was the capital of the state. The state lied between latitude 9.30N - 13N and longitude 27.4 E - 32E and was boarded six other states namely North Kordofan at the North and North East, South Kordofan at the East, Unity at the South East, North Bahr El Gazal at the south west, South Darfur at the West and South West, and North Darfur at the West and North West. (Fig 1). It consisted of six localities as shown in Fig 1.

In August 2005, and as a result of the Comprehensive Peace Agreement (CPA), West Kordofan State was abolished and its territory was divided between North and South Kordofan states, in implementation of the protocol between the Government of Sudan (GOS) and the Sudan peoples Liberation Movement (SPLM).
Fig (2-1) Former West Kordofan and neighboring States and area of study.

1- Anohoud.  
2- Lagawa.  
3- Kaliak.  
4- Abyei  
5- Asalam.  
6- Ghibiesh
The total human population was estimated as about one million (State Annual Report, 2004). The state was a home of complex ethnic groups, mainly Messeria, Hammar, Dinka, Nuba, and in addition to some other little groups from different tribes.

Climatologically, the state was located in the semi–arid belt, with increasing annual rainfalls from 300mm in the North to 600mm in the South. The local economy was comprised of mixed systems of crops and livestock production.

Livestock population density in West Kordofan State was well considered and the different component of livestock distributed all over the state, where sheep and camels were dominant in the northern part of the state while cattle were dominant in the southern part of the state.

In the Annual Report of Animal Resources Administration (2004) estimated livestock population to be:

- **Cattle** 3,839,600.
- **Sheep** 6,371,840.
- **Camels** 1,304,033.
- **Goats** 3,784,983.
- **Total** 15,300,456.

Seasonal livestock movements is mainly practised by Messeria sub tribes. They adopt cyclic seasonal movement of their herds depending on the rainfall, available water supply and the biting flies (Babiker, 1999). This regular pattern of movement is usually between North and South. It may extended to the grazing land in the neighboring southern states in the dry season.
2.2 Livestock types:

1- **Cattle:** The main types of cattle are Baggara zebu cattle, Foga, Kenana, and Nilotic types.

   The Baggara types is part of the shorthorned Zebu group of breeds of eastern Africa, but are smaller and thinner than the Boran breed of Kenya and Ethiopia. They are named after the Baggara people of western Sudan and central Chad, who keep Baggara cattle. Ironically, Baggara means cattle people in the Shuwa Arabic language of these people. The related Butana and Kenana breeds of the Nile Valley are dairy breeds and need much more feed and water than the Baggara, which are known for their hardiness and ability to thrive in the dry Sahel. The Baggara types have been improved by breeding projects of the Sudanese Department of Agriculture.

2- **Sheep types include:**

   1-Hammari.

   2-Garaj: Cross breed between Hammari & Nubian.

   3-Umbararo.

2.3 **Animal diseases in the study area:**

The main diseases which are controlled by annual vaccination in the study area are:

   1- Hemorrhagic septicemia. (HS).

   2- Black quarter. (BQ).

   3- Anthrax.

   4- Contagious bovine pleuropneumonia. (CBPP).

   5- Sheep pox.

   6- Peste de petite ruminants. (PPR).
The main diseases that can be controlled by medicinal treatment in the study area are:

1- Internal parasites (babesia, theleria, anapmlasma and worms.)
2- External parasite (ticks, mites and flies).

Other important diseases with no attempts for control include brucellosis, lumpy skin, and foot and mouth diseases.

Data about the prevalence of brucellosis in West Kordofan State are scarce, despite continuous complaints of animal owners about abortions.

They claim that: abortion in cattle usually occurs during the third trimester and they ignore the disease. No records are available about human brucellosis in the Ministry of Health in West Kordofan State.

2.4 Sterilization:

2.4.1-Sterilization of equipments:

Glassware such as test tubes, pipette, and Petri plates were sterilized in the hot oven at 160°C for one hour. Others like Bijou and universal bottles were sterilized in the autoclave for 15 minutes at 121°C.

2.4.2-Sterilization of culture media.

Culture media such as Brucella agar base, peptone water, and nutrient agar were sterilized in autoclave (121°C)

2.4.3-Sterilization of solutions:

Normal saline, phenol saline and distilled water were sterilized by hot air oven at 115°C for 10 minutes.

Universal bottles for collection of milk samples were sterilized by hot air oven at 120°C for 15 minutes.
2.4.4 Disinfection.

Seventy milliliters of absolute alcohol were mixed with 30ml of distilled water used for disinfection.

2.5 Collection of samples:

Samples used for detection of *Brucella* antibodies in cattle were blood, and milk. Hygroma fluids were used for the isolation of *Brucella* spp.

2.5.1 Blood samples:

Five hundreds blood samples were collected randomly from cattle of both sexes and of four age groups in fifty sites in the study area.

Distribution of samples according to sex for each locality is shown in Table (2-1).

The blood samples were collected aseptically from the jugular vein using 10 ml sterile syringes. Before introducing the needle of the syringe into the vein, the area was disinfected with 70% alcohol. After blood withdrawal, the syringes were put in an inclined position and kept about 24 hours at room temperature for separation of serum. The serum portion was then gently poured into disposable small sterile tubes and tested in the same day of collection for *Brucella* antibodies using Roes Bengal Plate Test (RBPT). All serum samples were then preserved in an ice box at and transported to the laboratory and kept in deep freezer (-20 °C) for further serological tests.

2.5.2 Milk samples:

A total of one hundred and twenty individual milk samples were collected from lactating cows in the main eight towns in the study area. Samples were collected in sterile universal bottles. Before sampling the
whole udder was washed with water and soap and dried, then the end of each teat was swabbed with 70% alcohol and wiped dry. The first one or two streams of milk were discarded.

Fifteen milk samples were collected from the different cattle types in the following towns, Alfula, Babanosa, Almuglad, Lagawa, Anohoud, Ghbiesh, Alkhowai, and Abuzabad.

2.5.3 Hygroma aspirates:

A total of twelve samples of hygroma aspirates were taken from 12 knee hygromas in baggara cattle from different herds in Almafoura, Alsonout and Al Arak (Lagawa locality). Aspirates were put in sterile bottles and taken to the lab in ice boxes for bacteriological culture.
Table 2-1 Blood collected from cattle for serum samples from both sexes and different places.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Total No. of samples collected</th>
<th>No. of samples from males</th>
<th>No. of samples from females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asalam</td>
<td>100</td>
<td>31 (31%)</td>
<td>69 (69%)</td>
</tr>
<tr>
<td>Gubeish</td>
<td>50</td>
<td>9 (9%)</td>
<td>41 (41%)</td>
</tr>
<tr>
<td>Kaliak</td>
<td>50</td>
<td>35 (35%)</td>
<td>15 (15%)</td>
</tr>
<tr>
<td>Abyei</td>
<td>100</td>
<td>26 (26%)</td>
<td>74 (74%)</td>
</tr>
<tr>
<td>Lagawa</td>
<td>100</td>
<td>9 (9%)</td>
<td>91 (91%)</td>
</tr>
<tr>
<td>Abuzabad</td>
<td>50</td>
<td>19 (19%)</td>
<td>31 (31%)</td>
</tr>
<tr>
<td>Anohoud</td>
<td>50</td>
<td>5 (5%)</td>
<td>45 (45%)</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>134 (26.8%)</td>
<td>366 (73.2%)</td>
</tr>
</tbody>
</table>
Figure (2-2) No. of blood samples for males and females in different localities.
Table (2-2) Blood for serum samples collected from different age groups of cattle.

<table>
<thead>
<tr>
<th>Locality</th>
<th>&lt;1 year</th>
<th>%</th>
<th>1-2 year</th>
<th>%</th>
<th>2-3 year</th>
<th>%</th>
<th>&gt;3 year</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asalam</td>
<td>17</td>
<td>17%</td>
<td>32</td>
<td>32%</td>
<td>14</td>
<td>14%</td>
<td>37</td>
<td>37%</td>
<td>100</td>
</tr>
<tr>
<td>Gubeish</td>
<td>4</td>
<td>8%</td>
<td>18</td>
<td>36%</td>
<td>13</td>
<td>26%</td>
<td>15</td>
<td>30%</td>
<td>50</td>
</tr>
<tr>
<td>Kaliak</td>
<td>6</td>
<td>12%</td>
<td>10</td>
<td>20%</td>
<td>12</td>
<td>24%</td>
<td>22</td>
<td>44%</td>
<td>50</td>
</tr>
<tr>
<td>Abyei</td>
<td>18</td>
<td>18%</td>
<td>27</td>
<td>27%</td>
<td>23</td>
<td>23%</td>
<td>32</td>
<td>32%</td>
<td>100</td>
</tr>
<tr>
<td>Lagawa</td>
<td>8</td>
<td>8%</td>
<td>11</td>
<td>11%</td>
<td>34</td>
<td>34%</td>
<td>47</td>
<td>47%</td>
<td>100</td>
</tr>
<tr>
<td>Abuzabad</td>
<td>3</td>
<td>6%</td>
<td>7</td>
<td>14%</td>
<td>13</td>
<td>26%</td>
<td>27</td>
<td>54%</td>
<td>50</td>
</tr>
<tr>
<td>Anohoud</td>
<td>5</td>
<td>10%</td>
<td>8</td>
<td>16%</td>
<td>6</td>
<td>12%</td>
<td>31</td>
<td>62%</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>12.2%</td>
<td>113</td>
<td>22.6%</td>
<td>115</td>
<td>23%</td>
<td>211</td>
<td>42.2%</td>
<td>500</td>
</tr>
</tbody>
</table>
Figure (3-3) No. of blood samples collected from cattle of different age group in different localities.
2.6 Serological tests:

Four serological tests were used for detection of *Brucella* antibodies in serum and milk. They were RBPT, SAT, ELISA and MRT. The reagents for all these tests were obtained from the *Brucella* Department, Central Veterinary Laboratory (CVL), Soba, Khartoum, except the antigen for ELISA was obtained commercially (Anigen 404–5 Woncheon, Yeongtong-gu, Suwon-si, Kyonggi, Korea 443). The AniGen B. *Brucella* Ab ELISA is indirect Enzyme Linked Immunosorbent Assay for the qualitative detection of *Brucella abortus* antibody in serum and plasma. The kit contains the following items.

1- Microplate coated LPS of *Brucella abortus* 1110-3:5 plates (96 wells/plate) configured in 12 strips.

2- Standard negative control serum: 1 vial of normal bovine serum treated with calcium. Sodium adize (0.01%) added as preservatives.

3- Standard strong positive control serum.

4- Standard weak positive control serum.

5- Sample diluents.

6- Washing solution

7- Enzyme conjugate and Enzyme conjugate diluent

8- Substrates A and B.

9- Stoping solution.

10- Adhesive plate sealer.

11- Instruction for use.
2.7 Tests procedures:

2.7.1 Rose Bengal plate test:

1- The serum samples and antigen were brought to room temperature (22±4°C); only sufficient antigen for the day’s tests was removed from the refrigerator.

2- A 25-30µl of each serum sample was placed on a white tile, plastic plate.

3- The antigen bottle was shaken well, but gently, and placed an equal volume of antigen near each serum spot.

4- Immediately after the last drop of antigen was added to the plate, the serum was mixed to antigen using a clean glass rod for each test.

5- The mixture was agitated gently for 4 minutes at ambient temperature.

6- Lastly, agglutination was read immediately after 4 minutes period is completed. Any visible reaction was considered positive.

2.7.2 Serum agglutination test:

Eight agglutination tubes were used in this study.

Amount of 0.8ml of phenol saline was placed in the first tube and 0.5ml in each succeeding tubes.

Then 0.2 ml from sample under test was transferred to the first tube and mixed thoroughly with the phenol saline.

Amount of 0.5 ml of the mixture was transferred over the second tube from which, after mixing, 0.5 ml was transferred to the third tube and so on until the last tube, from which 0.5 ml of the serum dilution was discarded. This process of double dilution results in 0.5 ml of dilutions
1:5, 1:10, 1:20 etc. To each tube was added 0.5 ml antigen at the recommended dilution and the contents of the tube were thoroughly mixed, thus giving final serum dilutions of 1:10, 1:20, 1:40, etc.

2.7.3 Milk ring test:

One ml of whole milk of each test sample was taken with sterile graduated pipette fitted with a teat rubber and put into an agglutination tube. Then 25-30µl of antigen (haematoxylin stained antigen) was added to agglutination tube. The milk /antigen mixture was incubated at 37°C for 1 hr, together with negative and positive control samples. Appearance of a blue ring in the cream layer at the top of the fluid column indicated a positive reaction.

2.7.4 ELISA test procedure:

Preparation of samples:

1- The serum samples under test were diluted 1:50 with sample diluents’. Then the microplate covered with adhesive plate sealer and mixed well on vibrating mixer. Mixing is very important to get the reproducible results. The plates were put in an incubator at 37°C for 60 minutes, washed five times with 350 µ of diluted washing solution and all liquid was aspirated from them.

2- About 100 µl of diluted enzyme conjugate was added to each well.

3- The microplate was covered with adhesive plate sealer. The wells were incubated at 37°C for 30 minutes, and washed five times with 350µl of diluted washing solution, and then all liquid was aspirated from the wells.

4- About 100µ of mixed substrate solution was added to each well, and the plates were incubated for 15 minutes at room temperature (18-25°C).
5- Then 100μ of stopping solution was added to each well.

6- Finally the absorbance of the wells read with a bichromatic spectrophotometer at 450 nm with reference wavelength at 620nm. Reading was completed within one hour from the end of assay.

2.8 Staining techniques

2.8.1 Preparation of smears.

Smears were prepared from hygroma fluid samples or by emulsifying a colony of *Brucella* culture on a clean dry glass slide. The smears were allowed to dry in air then fixed by gentle flaming.

2.8.2 Staining methods.

Gram stain was performed according to Cowan (1943), gram-negative organisms appeared red; this meant that the organism was gram-negative.

2.8.2.1 Modified Ziehl Neelsen stain

Smears were prepared from the hygroma fluid, dried and fixed over a flame, stained for ten minutes with a 1:10 carbol fuchsin (1 gm basic fuchsin dissolved in ten ml absolute ethanol solution), washed with tap water and differentiated with 0.5% acetic acid for not more than 30 seconds, washed thoroughly with tap water, differentiated lightly with 1% methylene blue (20 seconds). *Brucella* tube organisms were confirmed by the presence of organisms and blue background.

2-9 The isolation of *Brucella abortus* from a knee hygroma in cattle.

2.9.1 Primary isolation.

1. Twelve samples from hygroma fluids were used for isolation of *Brucella* organisms.
2. A drop from the positive hygroma fluid for RBPT was streaked on treptose agar plates.

3. The plates were incubated at 37°C in 10 percent CO2 atmosphere for 4 days.

4. All plates were examined on the third day of incubation and re-examined every other day for 10 days for organism resembling *Brucella*. Plates showing no growth or heavy contamination were discarded after 10 days. Only two isolates were obtained.

2.9.2 Sub culturing of primary isolate.

A typical and well isolated colony was picked up by loop and streaked on to a surface of *Brucella* medium plate then incubated.

2.9.3 Purification of culture.

Growth was checked for purity under the microscope by examining smears stained by modified Ziehl Neelsen stain.

2.10 Biochemical properties

2.10.1 CO2 requirement

The isolates were inoculated onto two SDA plates and incubated at 37°C for 2-3 days, one in air and the other in air +CO2 using a candle agar. Then the plates observed for growth.

2.10.2 Oxidase test

The test was performed by removing a portion of freshly grown culture with sterile glass rod and rubbed on a strip of filter paper, which was impregnated, with 1% solution of oxidase reagent. The immediate development of a dark purple colour indicated a positive reaction (Cowan, 1993).
2.10.3 Catalase test

The organisms were grown on nutrient agar. One ml of 3% hydrogen peroxide solution was added over the culture. Immediate production of gas bubbles was considered a positive reaction.

2.10.4 H2S Production.

The organism were grown on SDA slopes and lead acetate paper strip was inserted in the MacCarteny bottle without touching the medium then fixed in position by the bottle lid. The strips were examined daily for three days for blackening due to H2S production.

2.10.5 Urease production.

A heavy inoculum of the organism was seeded onto a slope of Christensen’s urea agar medium, incubated at 37°C (in 10% CO2) and examined at intervals of 15 minutes, one hour and then hourly for 24 hours. A positive reaction was indicated by the development of the purple pink colour (Cowan, 1993).

2.11 Biotyping of the organisms.

Biotyping of the species was carried out according to Alton et al. (1975 and 1988), using the following criteria: CO2 requirement, H2S production, growth in different concentrations of dyes and antigenic properties.

2.11.1 Growth in the presence of dyes.

SDA plates containing thionine and basic fuchsin at appropriate concentrations of (1:25000) to (1:100000), were streaked with a loopful of suspension of the strain to be tested. Suspensions were made by emulsifying a loop-full of each culture in 1 ml distilled water. Up to 5 suspensions cloud inoculated on each plate without touching each other.
The plates were inoculated as above after been labeled and plates were examined for growth after 3-4 days of incubation.

2.11.2 Antigenic properties (agglutination with monospecific Brucella antiserum).

Drops of A and B Brucella monospecific antisera were placed on a clean slide. To each drop was added an equal volume of the culture suspension to be tested. The drops were then mixed for about one minute and any agglutination recorded.
CHAPTER THREE

RESULTS

3.1 Results of serological tests:

The 500 serum samples were tested at Alfula veterinary regional laboratory and 24.6% were positive for the RBPT. The positive samples were tested by the SAT showed 23.6%. The titres ranged from 1:10 to 1:640. ELISA was used for confirmation of results in Central veterinary laboratory CVL at Soba. (Table 3-1) and 23% were positives. The MRT showed 5% positive samples (Table 3-4)

3.2 Prevalence of brucellosis in relation to herd structure.

The prevalence of brucellosis according to sex and age were illustrated in table (3.2) and (3.3).

3.3 Bacteriological findings:

3.3.1 The isolation and identification of Brucella organisms.

Examination of direct smears stained according to the modified Ziehl-Neelsen and Gram’s methods failed to reveal the presence of Brucella or other type of organisms. (Shigidi & Razig, 1971).

Br. abortus strains were isolated from two Hygroma samples. The isolates were identified on the base of their morphology and staining reaction, CO2 requirement, oxidase, and catalase as Br. abortus. The organisms were able to grow in presence and absence of CO2 and in Brucella medium with and without serum. They were non-motile and Gram’s stain showed Gram-negative coco-bacilli and the modified Ziehl Nielsen’s stain showed red coco-bacilli without bipolar staining. The organisms reacted with Br. abortus mono-specific antiserum.
3.3.2. Biotyping

All the strains from cattle in Previous West Kordofan State were identified and bio-typed as *Br. abortus* biovar 3. All were H2S and urease positive and grew in the presence of thionine in concentrations of 1/5000 and 1/100000.
Table (3-1) Results of the serological tests, RBPT, SAT, and ELISA in different localities.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Total samples collected</th>
<th>+ve samples for RBPT.</th>
<th>+ve samples for SAT</th>
<th>+ve samples for ELISA.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asalam</td>
<td>100</td>
<td>21</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Gubeish</td>
<td>50</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Kaliak</td>
<td>50</td>
<td>11</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Abyei</td>
<td>100</td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Lagawa</td>
<td>100</td>
<td>35</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Abuzabad</td>
<td>50</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Anohoud</td>
<td>50</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>123</td>
<td>118</td>
<td>115</td>
</tr>
</tbody>
</table>
Figure (3-1): Results of the serological tests, RBPT, SAT, and ELISA in different localities
Table (3-2) Rose Bengal Plate Test results of both male & female cattle serum sample from different localities.

<table>
<thead>
<tr>
<th>Localities</th>
<th>Total samples collected</th>
<th>Number of males</th>
<th>Result/ RBPT</th>
<th>Number of females</th>
<th>Result/ RBPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Asalam</td>
<td>100</td>
<td>31</td>
<td>7</td>
<td>24</td>
<td>69</td>
</tr>
<tr>
<td>Gubeish</td>
<td>50</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>Kaliak</td>
<td>50</td>
<td>35</td>
<td>6</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>Abyei</td>
<td>100</td>
<td>26</td>
<td>6</td>
<td>20</td>
<td>74</td>
</tr>
<tr>
<td>Lagawa</td>
<td>100</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>Abuzabad</td>
<td>50</td>
<td>19</td>
<td>3</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Anohoud</td>
<td>50</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>134</strong></td>
<td><strong>30</strong></td>
<td><strong>104</strong></td>
<td><strong>366</strong></td>
</tr>
</tbody>
</table>
Figure (3-2): RBPT results for both male and female in different localities.
Table (3-3) RBPT results of different age groups of cattle from different localities.

<table>
<thead>
<tr>
<th>Age group</th>
<th>&lt;year</th>
<th>1-2 year</th>
<th>2-3year</th>
<th>&gt;3year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Asalam</td>
<td>2</td>
<td>15</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td>Gubeish</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Kaliak</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Abyei</td>
<td>1</td>
<td>17</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Lagawa</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>29</td>
</tr>
<tr>
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<td>9</td>
</tr>
<tr>
<td></td>
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<td>24</td>
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<tr>
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<td>5</td>
<td>1</td>
<td>7</td>
</tr>
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<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>7 -12.9%</td>
<td>54</td>
<td>21 22.8%</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26 29.2%</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68 47.5%</td>
<td>143</td>
</tr>
</tbody>
</table>
Figure (3-3) Prevalence of Brucellosis in cattle according to age group in FWKS
Table (3-4) Milk ring test results:

<table>
<thead>
<tr>
<th>No.</th>
<th>Towns</th>
<th>Samples collected</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alfola</td>
<td>15</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Babanosa</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Almuglad</td>
<td>15</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Lagawa</td>
<td>15</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>Anohoud</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>Ghibiesh</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>Alkhowai</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>Abuzabad</td>
<td>15</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>120</td>
<td>6-5%</td>
<td>114</td>
</tr>
</tbody>
</table>
Figure: (3-4) Milk ring test results
4.1 Seroprevalence of brucellosis:

In this study, *Brucella* antibodies were detected in sera and milk by the four tests. RBPT, SAT, ELISA and MRT, and the results showed that the prevalence rate of cattle brucellosis by RBPT in Lagawa was the highest 35% followed by Anohoud and Ghibiesh 24% , Kaliak and Abuzabad 22% and the lowest were in Abyei and Asalam localities 21%.

The overall brucellosis prevalence was 24.6% by RBPT, but about 23.6% by SAT, and 23% by ELISA.

The findings of this study were in disagreement with the findings by the disease survey and map conducted in both North & South Kordofan states,(2007) which reported 85.9% prevalence. This disagreement might be for the bias samples collected from infected cattle with clinical signs in the disease survey and map.

On other hand the prevalence of this study showed that the disease wide spread compared with earlier results of Daffalla (1962) who had reported the prevalence 15% in South Kordofan and 8.7 -10.7% in Gazira in central Sudan, Abdalla (1964) 3% in Northern Sudan, El Nasri (1960) 12.6-18%, Mustafa and Hassan (1969) 5.7-8.7% prevalence rate in Blue Nile State, Ibrahim and Habiballa(1975) in Messeriya cows found 14.2 and 66.7% and Raga (2000) and Musa (1995) reported 6.2% 13.9% in Darfur States, respectively.
4.2 Brucellosis in relation to herd structure.

The prevalence rate increases with age Table (3-5) this in agreement with Agab et al., (1995) who reported similar observations that the prevalence of the disease increases with age.

Percentage of infected male cattle was high about 28.8% in PWKS and the percentage of infected females was about 34%. Rankin suggested that the infection of males occurs at a very young age and it is not only very rare that infected bulls could spread the disease to cows during natural service. He also added that brucellosis is not an important cause of infertility in bulls. Since western baggara bulls are not used for artificial insemination, the economic importance comes in rejection of consignments containing brucellosis reactor bulls.

4.3 Isolation of Brucella:

*Brucella abortus* was isolated from cattle and all isolates were identified as Brucella abortus biovar3. This finding is in agreement with those from Darfur states, Raga (2000) isolated the same biovar from cattle. Open borders with neighboring states crossing by cattle owners in search for good pasture results in the contamination of the environment and transmission of the organisms between animals. Also these results are in agreement with that of Adil (1987) who identified his isolates as *Brucella abortus* biovar 3, Agab et al., (1995) isolated the same biovar from camels which were mixed with cattle in Butana area. On the other hand, this finding is disagrees with the results obtained by Musa(1995) who identified and biotyped all his isolates from Darfur states as *Brucella abortus* biovar 6 and he suggested that, this biovar was the major cause of cattle brucellosis in Sudan.
All isolates in the present study were obtained from cattle with hygromas and Hygroma fluids were found to be good sources for isolation of *Brucella* organisms. This is in agreement with the observation of Domench et al (1984) who found that 60% of Brucella stains isolated from cattle were from hygromas. Most of the cattle herds investigated in this study were reared in nomadic or semi-nomadic, but few were in sedentary areas.

**4.4 Recommendation:**

On the bases of this study, it appears that control and eradication programmes of brucellosis should be adopted. The system of animal husbandry practiced in PWKS makes control and eradication of the disease difficult. However, to minimize the spread of this zoonotic disease the following measures are recommended.

1. Education of animal owners how to get rid of aborted fetuses and placentas of aborted animals.
2. A mass animal vaccination of all species.
3. Systemic surveys to monitor the disease situation.
4. Attempts should be made to minimize the density of animals in watering places by increasing the number of watering places.
5. Other preventive measures by government such as control of animals movement across the borders should be followed.
6. More extensive surveys to determine the size of the problem of
7. brucellosis in all livestock species in the different part of the Sudan
8. Determination of the proper age of vaccination and duration of immunity in local cattle.
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