CHAPTER ONE
LITERATURE REVIEW

1.1. Definition of Brucellosis

Brucellosis is a highly contagious zoonotic, and economically important bacterial disease of animals worldwide (OIE, 2000). It is a serious problem of domestic animals, especially cattle, sheep, and goats and wild causing a decrease in reproductive efficacy and an increase abortion rate (Rijpens, et al., 1996). The disease is caused by gram negative facultative, intracellular, bacterial organisms of the genus *Brucella* that are pathogenic for a wide variety of animals and human beings (CDC, 2005).

1.2. The Importance of Brucellosis

The importance of brucellosis arises from the fact that it is highly contagious to human. Moreover, the disease has economic impact on the animal industry, causing an adverse effect on animal health. According to (FAO, 2003) it is the second most important zoonotic disease in the world after rabies. The disease is mainly considered as having high risk to exposed professionals such as veterinarians, farmers, laboratory technicians, abattoir workers, and others who work with animals and their products. The prevalence of human brucellosis acquired from dairy products is seasonal, reaching a peak soon after kidding and lambing. Brucellosis is still a major problem, widely distributed throughout the world, mainly in developing countries due to traditional feeding habits and the failure to maintain standards of hygiene because of socio-economic conditions (Ozekicit et al., 2003).

1.3. Etiology

Brucellosis is caused by different species of the genus *Brucella* which are pathogenic to a wide variety of animal and also to human. *Brucella* are gram-negative bacteria, small, non spore forming, non encapsulated, non-motile, cocco bacilli (Corbel and Morgan 1984). They are usually arranged
singly, or in pairs, or small groups. They are intracellular organism and can usually be found in the reticulo endothelial and reproductive system (Alton et al., 1975). They are not truly acid-fast but resist decolouration by weak acids, thus they stain red by the Stamp's modification of Ziehl-Neelsen method. The morphology of Brucella is fairly constant except in old cultures, where pleomorphic forms may be evident (Alton et al., 1988). The genus Brucella is species (spp) specific that tend to infect specific animal species but most of Brucella spp can infect other animal species and some are zoontic Brucella. Spp. have the ability to escape the host defense mechanism and survive and replicate inside the cell (Youg, 1995).

1.4. Taxonomy

All members of the Brucella genus are closely related, the variation, based on relevant differences in host preference and epidemiology displayed by the major variants, as well as molecular of genomic variation may occur. (Verger et al., 1985) considered the high degree of deoxyribonucleic acid (DNA) homology for all species as that all types of Brucella spp should be regarded as biovars of Brucella Melitensis, however this assumption has not yet met with complete agreement.

The classification used world-wide is the old classification which split the genus into six species Brucella abortus, and its main hosts are cattle and camels, Brucella melitensis, the main hosts are sheep and goats, Brucella suis the main host is pig, Brucella neotomae infect only desert wood rat, Brucella ovis infect sheep and Brucella canis for dog (Moreno and Moriyon 2001). A marine species has been noted Brucella maris for marine mammals, and two other new species, Brucella ceti and Brucella pinnipedialis have been identified (Sohn, et al. 2003). A new strain, named Brucella microti, was recently isolated from the common vole (Microtus arvalis) in Central Europe (Lopes et al., 2010).
The first three *Brucella abortus, Brucella melitensis* and *Brucella suis* are recently subdivided into biovars based on cultural and serological properties (OIE, 2009).

1.5. Historical Background

The first discovery of *Brucella melitensis* as the cause of Malta Fever from the spleens of soldiers dying of Mediterranean fever by Bruce in 1887 (Alton, 1990). The isolation of *Brucella abortus* from aborted cattle by Bang in 1897 (Mcmann, 1944).

In Sudan Animal brucellosis was discovered as early as 1904 and was first reported by Bennet (1943) in Khartoum. Later many researchers surveyed the disease in different animal species in different localities in Sudan historically. The rates of positive reactors in goats were 2.5% -5.9% in the Gezera (Dafalla and Khan, 1958), 5.7% -8.3% in the Upper Nile Province (Nasri, 1962), 1.5% in Wadi Halfa (Abdallah, 1966). However, the prevalence rate in cattle is reported in all parts of Sudan and was found to be higher than other animal species.

1.6. Pathogenesis

Infection by *Brucella* varies and affected by the size of the infective dose and virulence of the bacteria. A fully virulent *Brucellae* are highly invasive and capable of penetrating the mucosa of the nose, throat, conjunctiva, urogenital tract, teat canal, and abraded skin (Davis *et al.*, 1990). The resistance of the animal vary according to age, sex, and the reproductive status of the animal (Nicoletti, 1980). The normal route of infection is through the oral route by licking aborted fetus, infected placentas, and vaginal discharges or by ingestion of contaminated feed and water. The bacteria enter the body through penetration of the mucous membranes of the alimentary tract, survive and multiply in cells of the mononuclear phagocytic system (Herr 1994, Godfroid *et al.*, 2004 a). After penetration, the organisms are phagocytosed by the neutrophils and macrophages and carried to the regional lymph nodes. The organisms multiplies leading to lymphadenitis.
and these may be followed by bacteraemia (Radostits et al., 1994, Godfroid et al., 2004b).

During bacteraemia the organisms are carried intra cellularly or free in the plasma then will be localized in organs like pregnant uterus. In acute cases, up to 85% of the bacteria are in cotyledons, placental membranes, and allantoic fluid, udder, supra mammary lymph node and the spleen (Radostits et al., 2000). In non-pregnant cows the organisms localized in the udder and uterus, and in cases where the animal becomes pregnant bacteremic phases occur in the udder. Infected udders are clinically normal but they are important as a source of infection of the uterus and also a source of infection to calves and humans by drinking the infected milk (Corbel, 2006). In male the testes and male accessory sex glands are infected (Godfroid et al., 2004a). Abortion is typically the first clinical sign of the pregnant female, and orchitis and epididymitis are typical clinical sign of the male (Corbel and Macmillan, 1998).

Infection by Brucella melitensis in sheep and goats resembles infection by Brucella abortus in cattle however, the udder is an important predilection site for Brucella melitensis. Greatly reduced milk yield follows abortion, and infection of the udder following a normal birth also leads to a considerable reduction in yield. In spite of this, clinical signs of mastitis are seldom detectable in naturally infected goats as well sheep that abort often excrete the bacteria in the milk (Alton, 1990).

1.7. Clinical Signs

1.7.1. Clinical signs in sheep and goats

Brucella melitensis is the main cause of abortion in goats and sheep and Malta fever in humans. Brucella melitensis mainly causes abortions, stillbirths and the birth of weak off-spring, and there may be retention of the placenta in aborted animal. The abortion usually occur only once, but reinvasion of the uterus and shedding of organisms can occur during next subsequent pregnancies. Milk yield is significantly reduced in animals that
abort and in animals whose udder becomes infected after a normal birth. However, clinical signs of mastitis are uncommon. Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes. Many non-pregnant sheep and goats remain asymptomatic (DelVecchio et al., 2002).

*Brucella ovis* is also an important cause of orchitis and epididymitis in sheep, but it is not recognized as a cause of infection in goat (Smith, 1996).

1.7.2. Clinical signs in cattle

Brucellosis in cattle is usually caused by biovars of *Brucella abortus*. Infection can also be caused by *Brucella Melitensis* (Verger, 1985). *Brucella suis* may cause a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion or spread to other animals (Ewalt et al., 1997). In cattle, *Brucella abortus* causes abortions, stillbirths and weak calves; abortions usually occur during the second half of gestation, and there may be retention of placenta and reduction of milk yield. After the first abortion, subsequent pregnancies are generally normal; however, cows may shed the organism in milk and uterine discharges. Epididymitis, seminal vesiculitis, orchitis and testicular abscesses are sometimes seen in bulls.

1.7.3. Clinical signs in camel

The main cause is *Brucella abortus* and *Brucella melitensis*. The association between Brucella infection and abortion in camels has been established (Higgins 1986; Agab and Abbas 1999). The clinical signs of brucellosis in these animals are similar to those in cattle.

1.8. Diagnosis of Brucella

The clinical signs of the disease are not pathognomonic, although the herd history may be helpful and hence, laboratory diagnosis is required for identification and elimination of infected animals. There is no single test by which a bacterium can be identified as *Brucella*. A combination of growth characteristics, serological, bacteriological and/or molecular methods is usually needed, (OIE, 2009). However, the most accurate diagnosis of the
disease can be made only by the isolation and identification of *Brucella* from abortion material, udder secretion or from tissues removed at post-mortem (OIE, 2009). But in situations where bacteriological examination is not practicable, diagnosis of Brucella infection must be based on serological methods (Alton *et al.*, 1988).

### 1.8.1. Bacteriological methods

These methods include isolation by culture media and/or identification by staining methods, however due to the biological properties of *Brucella* the cultural methods are time-consuming and when the level of infection is low they may fail (Gallien *et al.*, 1998; Fekete *et al.*, 1990). The samples could be milk, essentially from all quarters, vaginal swabs, blood culture, aborted materials e.g. fetal membranes and aborted fetus, and also animal carcasses for reticulo-endothelial tissues. The materials for laboratory examination should be cooled immediately and transported to the laboratory (Alton *et al.*, 1975).

### 1.8.2. Serological methods

They play a major role in routine diagnosis of brucellosis. These detect the presence of antibodies in the samples collected from animals, however the presence of antibodies does not always mean an active case of brucellosis, since post vaccination immune responses also has the same, and other Gram negative bacteria such as *Yersinia enterocolotica* may cross-react with smooth *Brucella spp.* (Corbel and Hendary, 1983).

The most common important serological tests which are used in diagnosis of brucellosis are buffered Brucella antigene (BBA) such as Rose Bengal Plate test (RBPT) and serum agglutination test (SAT). RBPT is used as a screening test, however the positive sera must be tested by confirmatory test because it gives high proportion of false positive reactors. SAT is most useful in human and bovine brucellosis. The test is used for control programmes and import and export policies as it has an international standardization. Complement fixation test (CFT) is used for confirming the results of the
RBPT and SAT are considered the most accurate serological method for diagnosis in cattle, sheep, goats so it is recommended by World Organization for Animal Health (OIE) as a test prescribed for international trade, but it’s complicated method to perform requiring good laboratory facilities and trained staff. However, it’s sensitivity and specificity are limited and it should be regarded as a complementary rather than confirmatory test.

Milk ring test (MRT) can be used with milk from individual animals or bulk milk samples hence, this test is valuable for detecting infected herds (Alton et al., 1975). The MRT is not suitable for use on sheep or goats milk.

Enzyme-linked immuno- sorbent assays (ELISAs) are most sensitive and specific however, unlike C-ELISA the I-ELISA are not capable of fully resolving the problem of differentiating between antibodies resulting from S19 vaccination (Alton et al., 1988; Morgan, 1982). ELISA is reported to give superior results to other tests in sensitivity and specificity (Corbel, 2006).

The serological tests used for cattle are applicable to camels. In sheep and goats The RBPT is useful for screening sheep and goats sera for antibodies to Brucella melitensis. However, it is less sensitive than in cattle and may not detect some infected animals hence, it is best used in combination with the (CFT).

1.8.2.1. Rose Bengal Plate Test (RBPT)

The RB antigen is a suspension of Brucella abortus smooth culture cells stained with the Rose Bengal dye, buffered to pH 3.65. The test is based on mixing a drop of serum and a drop of antigen together. It is important to note that the antigen has to be shaken before it is used. It must not freeze, and should be stored at 4 °C in a dark place. The RBPT is very sensitive, commercially available and rapid screening method (EC, 2002) however, like all other serological tests, it could sometimes gives false positive result because of Brucella. abortus strain 19 vaccination( S19)or of false-positive serological reactions (FPSR). Therefore, positive reactions requires confirmation by some other more specific test like CFT and ELISAs.
Nevertheless RBPT appears to be adequate as a screening test for detecting infected herds or to detect the absence of infection in brucellosis-free herds. (OIE, 2009).

1.8.2.2. ELISA tests

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in a kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosing a wide range of animal and human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted however, that although the ELISAs are more sensitive than the RBPT, sometimes they do not detect infected animals which are RBPT positive. Some protocols are less sensitive than others, therefore results obtained from different assays are not always comparable. It is also important to note that ELISAs are only marginally more specific than RBT or CFT (Corbel, 2006). In this test, Brucella antigen is bound to a solid phase, usually a polystyrene microtitre. Following that, the serum under test and a monoclonal antibody directed against an epitope on the antigen are co-incubated. This anti-brucella monoclonal antibody is conjugated to an enzyme, the presence of which is detected if it binds to the antigen and gives coloured reaction. This will only occur if there is no antibody in the serum sample which is bound preferentially (Nielsen et al., 1991).

1.8.3. Delayed immuno-hypersensitivity reaction tests

This procedure, using a standardized antigen preparation such as Brucellin INRA (an antigen (LPS-free extract) which is prepared from a rough strain of Brucella. Melitensis). It can be used for monitoring the status of herds in brucellosis-free areas. It is sensitive and has a very high...
specificity, such that serologically negative unvaccinated animals that are positive reactors to the brucellin test should be regarded as infected animals. However false positive reactions can occur in vaccinated animals. (Saergerman et al., 1999; Pouillot et al., 1997).

1.8.4. Molecular Biology

Leal-Klevezas et al., (1995) presented a method for the extraction of Brucella DNA from serum and milk samples of infected animals. Polymerase chain reaction (PCR) is a reliable tool for the detection of Brucella spp. from body fluids of infected animals. It is highly sensitive, very specific, rapid and reliable (Bricker, 2002). The test can detect a few bacteria in a sample also can detect dead bacteria reducing the necessity to conserve sample before analysis. PCR can provide both a complementary and biotyping method based on specific requirements. The sensitivity of the test and its ability to detect the pathogen in samples from the field reveal a promising advance in the diagnosis of brucellosis in animals and humans.

1.9. Epidemiology

1.9.1. Distribution

Bovine brucellosis is usually caused by Brucella abortus, less frequently by Brucella melitensis, and infection may occur occasionally by Brucella suis. Infection is widespread globally (OIE, 2009). Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are considered to be free from the agent (Geering et al., 1995). The disease in cattle has been recorded in 120 out of 170 countries (Nielsen and Dunkan, 1990). Though its distribution is worldwide, yet brucellosis is more common in countries with poorly standardized animal and public health programme (Capasso, 2002). The prevalence in Arabian countries vary from (1.30%) in Emirates (Afzal and Sakkir 1994) to (23.30%) in Egypt (Refai, 1989).

Brucella melitensis is the main causative agent of brucellosis in sheep and goats, the disease in sheep and goats is restricted to the Mediterranean
region, west and central Asia and Arabian peninsula, South America, Africa and India (Nielsen and Duncan, 1990). Northern Europe and North America (except Mexico) is believed to be free, (except for sporadic incursions from the south) (Godfroid and Kasbohrer (2002), (Pappas et al., 2007), Southeast Asia, Australia and New Zealand (OIE, 2009).

Brucellosis in camels seems to be widespread among camel herds in Africa and on the Arabian Peninsula. The prevalence of brucellosis in camels has been reported from Arabian and African countries and vary from (0.0) in some parts of Sudan (El-Ansary et al., 2001) to 17.20% in Iraq (Al-Ani et al., 1998).

The main factors influencing the epidemiology of brucellosis in animal are classified into factors associated with the transmission of the disease among herds (movement of animals, and the proximity to infected herds, vaccination level) and the factors influencing the maintenance and survival of the bacteria and spread of infection within herds (husbandry practices, herd size, population density and the method of housing and contamination from wildlife. (Crawford et al., 1990).

1.9.2. Transmission

*Brucella spp.* are usually transmitted between animals by direct contact with the placenta, fetus, fetal fluids and vaginal discharges from abortion or full term parturition. Although ruminants are usually asymptomatic after their first abortion, they can become chronic carriers, and continue to shed *Brucella* in milk and uterine discharges during subsequent pregnancies. Entry of *Brucella* into the animal body occurs by ingestion and/or through the mucous membranes of animal (broken skin and possibly intact skin). (Quinn et al., 1994). In vertical transmission *Brucella abortus* infection in cattle and, *Brucella melitensis* can be transmitted from the mother to their newborns or kids. The majority of infections are probably acquired by consumption of colostrum or milk. These newborns or kids may have infections in the lymph nodes draining the gastro-intestinal tract and may shed
organisms in the faeces. It is also probable that a self-cure mechanism takes effect in most of the infected kids. Similar to Brucella abortus infection in cattle, Brucella melitensis can be transmitted from the dams to lambs or kids (Grilló et al., 1997).

Brucella species infections are characterized by a marked preferred animal host specification, the primary hosts act as reservoirs of infection for each particular species, while the secondary ones usually play little part in the maintenance or spread of the disease (Corbel and Hendary, 1983).

Most Brucella species are also found in semen. Males can shed these organisms for long periods or lifelong. The importance of venereal transmission varies with the species and is apparently important only in swine Brucella suis and Brucella ovis infection in sheep. Sharing of breeding stock males between farms seem to promote transfer of infection between farms (Alton, 1985; Mikolon et al., 1998). The infected bulls used in natural service do not play a major role in spreading the infection. However in artificial insemination because the infected semen is ejaculated into the uterus it may spread the disease (Bendixin and Blood, 1947).

Brucella species have also been detected in other secretions and excretions including urine, feces, hygroma fluids, saliva, and nasal and ocular secretions. In most cases, these sources seem to be relatively unimportant in transmission. Brucella can be spread on fomites including feed and water in conditions of high humidity, low temperatures, and no sunlight. Wild carnivores and dogs present special risk to intensively managed livestock and their human owners as they carry the aborted material to clean areas. (Nicoletti, 1980).

Human to human transmission is rare, but has been reported Examples of human-to-human transmissions by tissue transplantation or sexual contact are occasionally reported but are insignificant (Mantur et al., 1996). Common sources of infection for people include contact with animal abortion products, ingestion of unpasteurized dairy products from cows, small ruminants or
camels, or other uncooked meat products. Also contact with laboratory and tissue samples or cultures can aid in the transmission (Schnurrenberger et al., 1975; Stableforth and Galloway 1959.)

1.9.3. Survival of *Brucella* species

*Brucella* species are intracellular organism. The external membrane component of *Brucella*, which is lipopolysaccharide (LPS), has a unique structure that afford it with a very low endotoxicity, hence resist the host immune response and confers resistance to antimicrobial activity and acts as virulence factor for survival and intracellular replication (Lapaque et al., 2005). In the environment the ability of *Brucella* to persist outside mammalian hosts is relatively high compared with most other non-sporing pathogenic bacteria, under suitable conditions. *Brucella* can survive drying, particularly when organic material is present, and survive in dust and soil. Survival is longer when the temperature is low. *Brucellae* are sensitive to direct sunlight, disinfectants and pasteurization. The organism is killed by pasteurization or complete exposure to Ultra violet (UV) or Gamma rays (King, 1951). Environment is not considered an important source of infection (Wray, 1975).

1.9.4. Risk factors for transmission

The factors affecting the transmission of *Brucella* could be classified to two:

1-Factors affecting transmission between herd like movement of the animal from an area to another so the disease can spread from infected herd to non infected herd (Radostits et al., 1994). Also sharing the same pastures is a way of infection where infected animals mix with uninfected ones or get in touch with contaminated premises, manure. Introducing new infected animal to uninfected herd can also aid in the transmission of disease.

2-Factors affecting transmission within the herd and these include density of animal populations, the herd size, the type and breed of animal (dairy or beef), the type of husbandry system and other environmental factors are thought to be important determinants of the infection (Salman and Meyer, 1984).
From epidemiological evidence, *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* have distinct host preferences. The organisms are capable to cause an infection in a wide range of host species, including humans so frequent isolation of *Brucella melitensis* from camels refer to contact with sheep and/or goats (Abbas et al., 2000). The remaining three members of the species have much greater host specificity. Herd size was identified as a risk factor for brucellosis in camels. These observations were reported previously by (Abbas and Agab, 2002). Larger herds provide more chances of contact between camels, especially during the calving season.

Breed may also affect susceptibility, most breeds of goats are fully susceptible to infection and a great variation in the susceptibility of different breeds of sheep has been reported. The milking breeds seem to be the most susceptible to *Brucella melitensis* (Corbel and Brinley-Morgan, 1984). Breed differences in susceptibility have not been clearly documented in cattle although genetically determined differences in susceptibility of individual animals have been demonstrated.

In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature animals. Young animals are often resistant, this resistance is due to resistance of sexually immature animal to infection, which become susceptible to disease with age (Paul, 1980), due to passive immunization of off spring through colostrum of their infected dams. However, it should be noted that latent infections can occur and such animals may present a hazard when mature (Radostits et al. 2000). As sex hormones and erythritol tend to increase in concentration with age and sexual maturity and favor growth and multiplication of Brucella organisms (Radostits et al., 2007). However, some reports indicate that Brucella antibody titers are not associated with sex (Muma et al., 2006).
1.10. Prevention

It is more economical and practical to prevent diseases than to attempt to control or eliminate them. For brucellosis, the measures of prevention include:

1- Attention and careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from clean herd or flocks. Serological screening tests are necessary.
2- Isolation of purchased replacements for at least 30 days.
3- Prevention of contacts with herds of flocks of unknown status or those with brucellosis.
4- If there are cases of abortions, premature births, or other clinical signs suspected animals should be isolated until a diagnosis can be made.
5- A periodic milk ring tests in cattle (at least four times per year),
6- Testing animals with simple screening serological procedures such as the RBPT and slaughter the positive reactors.
7- Proper disposal of placentas and dead fetuses and disinfection of contaminated areas should be performed thoroughly (Corbel, 2006).

1.11. Control

Because brucellosis is a disease of major economic and public health importance, a strategy for its control is essential in endemic areas. The initial aim of the strategy selected will be the reduction of infection in the animal population to an acceptable level so that the impact of the disease on human health as well as on animal health and production will be minimized (Kaplan, 1966). The second steps must include eradication from a region by test and slaughter and, following successful eradication, measures to prevent reintroduction of the disease should be applied.

An effective control of animal brucellosis requires the following basic elements: (1) Surveillance to find all the infected animals and herds which need an adequate veterinary organisation and laboratory testing to be available, (2) Controlling the transmission of the infection to new animals or
herds by the control of movements of animals. But according to Corbel (2006) it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries, hence co-operation of the animal owners with the existing programme is helpful and (3) The eradication of the reservoir to eliminate the sources of the infection in order to protect susceptible animals or herds (Metcalf, 1986).

1.11.1. Vaccination.

Vaccination is often the first step in the control of infectious diseases like Brucella. The most commonly used vaccines are Brucella abortus S19 and Brucella melitensis Rev.1 vaccines. Brucella abortus RB51 vaccine is used in some countries on small scale (Refai, 2002). Administration of currently available vaccines alone is not sufficient for elimination of brucellosis in any host species. Development of safer and more efficacious vaccines alone, or combined with enhancements or increased emphasis on other regulatory program components, could have tremendous impact on reducing the worldwide prevalence of brucellosis (Olsen and Stoffregen, 2005). Blasco (1997) reported that the live Brucella melitensis Rev 1 strain is considered as the best vaccine available for the prophylaxis of brucellosis in small ruminants. But the vaccination of pregnant animals with full standard doses of Rev 1 administered sub cutaneously is followed by abortion in most vaccinated animals and also may lead to persistent serological responses as indicated by the classical methods of serological diagnosis. This will inevitably interfere with an eradication programme based on a test-and-slaughter policy and accordingly a reduced-dose vaccination strategy has been widely used and has been reported as safe. However, reduced doses of Rev 1 should not be recommended as an alternative to the full standard doses. For sheep, conjunctival administration of standard doses of Rev 1 during the late
lambing season or during lactation is recommended as a whole-flock vaccination strategy.

*Brucella abortus* strain 19 vaccine is the most widely used vaccine to prevent bovine brucellosis it has low virulence of causing abortion except if the cows are vaccinated in late pregnancy. This vaccine can cause undulant fever in humans and in many cases it fails to prevent infection completely especially infection of the udder and the persistence of vaccinal titers in some animals. Vaccination of bulls result in the development of orchitis and presence of *Brucella abortus* strain 19 in the semen so it is discouraged (Radostits *et al.*, 2006).

**1.12. Treatment of Brucellosis**

In vitro nearly all *Brucella* strains are sensitive to gentamcin, tetracycline and rifampin (Corbel and Brinley- Morgan, 1984), but in fact because of the intracellular characteristics of *Brucella* which determine the chronic course of the disease and its tendency to relapse , antibiotic treatment of known infected animals, or of those which are potentially exposed to them, has not been commonly practiced. Treatment should be ruled out as an option in the control of brucellosis and according to Corbel (2006) treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended. However, the course of the disease may be modified by tetracycline alone or in combination with streptomycin. Effective controls must be based on minimizing the infection by improve the sanitary methods, control the factors that help in the spread of the disease, and a vaccination program (Fensterbank, 1976 ; Nicoletti and Milward, 1985). A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd or flock was treated but this procedure is considered to be restricted in practice. According to Radwan *et al.*, (1987) a long term treatment with a high dose of oxytetracycline (1000 mg/day for 6 weeks, I/P) had completely eliminated *Brucella melitensis* from naturally infected sheep.
1.13. Economic Impact

Food and Agriculture Organization of the United Nations (FAO) and the Organization of Animal Health (OIE) consider the importance of this disease. Brucellosis has not only direct public health implications but also poses a potential barrier to international trade of animals and animal products. Such a barrier could seriously impair socio-economic development, especially in rural populations - the livestock owner (WHO, 1997). The disease has a considerable impact on animal and human health, as well as socioeconomic impacts, especially in areas where the rural income depends on livestock breeding and dairy products. It is one of the most serious diseases in developing countries. The rates of infection vary greatly from one country to another and between regions within a country.

The economic loss from brucellosis in developed countries arises from the slaughter of cattle herds that are infected with brucellosis and all the cost of eradication and control program. Farmer in developing countries suffer from the actual abortion of cows and the decreased in milk yield, birth of weak calves that die soon after birth, retention of placenta, impaired fertility and sometimes arthritis or bursitis and all the cost of tests and samples. Death may occur as a result of acute metrites (Radostits et al., 2000). The loss in a developing country is due to prophylactic activities, control and eradication program, hospitalization of human patients, cost of research, loss of work or income and failure in financial investment (Chukwu, 1987). Also the restriction of international trade in animals and their products constitute a major economic loss (Corbel, 1973).

The main point in quantification of the financial effects of animal diseases is to make decision to know the best way of disease control measures based on costs and benefits (Chilonda and Huylenbroeck, 2001). The quantification of the losses due to individual animal diseases depends on the disease investigation work undertaken. Once the actual disease prevalence and the nature and magnitude of the losses tested in infected herds at the regional
and national levels have been defined, the economic portion of the analysis can be accomplished by:

1- Organize and classify the information on disease losses.
2- Quantify the losses, choosing prices that reflect the economical of the analysis being undertaken. Depending on the information available the estimation of the annual level of losses associated with the disease can be made by estimating the value of the animal and the effect of disease on the final output.
3- Attempt to quantify the indirect losses attributable to a disease (Put et al., 1988).

To estimate the financial loss caused by brucellosis, it depends mainly on the type of cattle farming, herd size, and loss in reproduction in meat and milk due to abortion. The infected non aborting dairy cows produce 10% below potential and aborting 20% (Crawford et al., 1978). The percentage of abortion in infected cows annually is 10-35% (Shepherd et al., 1979).

The disease causes heavy economic losses in small animal production resulting from abortions, abortion rated up to 50% in sheep and goats have been reported by Nicoletti (1982). Sterility, decreased milk production, and the costs of replacer animals and the effect of the disease on ram fertility can influence the number of rams required in a flock. Lambing percentage is reduced by 30% in flock recently infected and by 15-20% in endemic infection (Ariza et al., 1992 and Radostitis et al., 2000). In addition, the disease is an impediment to free animal movement and export.


1.14.1. Small ruminant brucellosis sheep and goats
In Palestinian Authority the highest rate of infection in animals (72.9%) was reported by Shuaibi (1999). The samples were taken from flocks suspected of being infected due to abortions or the presence of human cases. In Ethiopia Ashenafi et al., (2007), Yesuf et al., (2010), Ferede et al., (2011) and
Tesfaye et al., (2012) reported 3.2%, 2.5%, 1.2% and 3.8% prevalence rate in small ruminants respectively. In Eritrea according to Omer et al., (2000) the individual prevalence 3.8% in goats and 1.4% in sheep while the unit (herd) prevalence is 33.3% in goats and 16.7% in sheep in the eastern part of Eritrea. On the other hand the individual prevalence rate in goats was 14.3% and the units prevalence was 56.3% in the western Eritrea.

In Yemen, sera from 538 Yemeni goats and 690 Yemeni sheep were screened for brucellosis by the RBPT and the reactors were confirmed by the CFT and the SAT (Hosie et al., 1985). The prevalence among goats was 0.4% and among sheep was 0.6%. In Nigeria Cadmus et al., (2006) reported 0% prevalence in sheep (0/54) while Bertu et al., (2010) reported 14.5% prevalence rate in small ruminants. Akbarmehr and Ghiyamirad (2011) reported 4.2% prevalence rate in small ruminants in Iran. In the United Arab Emirates sero-prevalence survey of brucellosis in livestock including sheep and goats was conducted by Mohammed et al., (2013) in different regions of Abu Dhabi Emirate. They used RBPT as screening test and confirmed their result by C-ELISA. The overall sero-prevalence of Brucella antibodies was 8.00%.

In Sudan El-Ansary et al., (2001) studied the relative frequency of brucellosis among domestic animals in Kassala State, Sudan, in the year 1999. Sera of animals brought for slaughter to Kassala abattoir and sera of occupational contacts of animals were collected. A total of 1038 sera were tested by the slide agglutination test. The positive reactors were confirmed by tube agglutination test. 4% of goat’s sera, 1% of sheep sera were found positive. Omer et al., (1989 to 1990) screened 33,591 castrated sheep males that were ready for export from (Alkdr) quarantine, Khartoum State and Portsudan quarantine, Red sea State by RBPT. The prevalence rate of sheep brucellosis was 0.01%. Omer et al., (2007) studied the prevalence of brucellosis in Kassala, Eastern Sudan, during (2004-2006) the result was that: prevalence rate was 0.1%, 0.4%, 2.1% in sheep and 0.2%, 0.6%, 5.6% in
goats in the three years respectively. Musa (2005) investigated sheep brucellosis in Darfur States and reported a prevalence rate of 3.3%. Omran (2011) investigated the disease in Sinnar State and got 4.1% prevalence rate in 585 heads of sheep. She used modified RBPT, SAT and ELISA. According to Ali (2013) the seroprevalence was 2.5% by RBPT in sheep in North Kordofan (8/318). A prevalence of 2.0% in goat in Khartoum was reported by Magzub (2001).

### 1.14.2. Cattle brucellosis

In Iran the prevalence was 3.66% based on Akbarmehr and Ghiyamirad (2011). In Kampala, Uganda, the individual prevalence of bovine brucellosis was 5.0%, using (C-ELISA). Large herd size and history of abortion are significant risk factors (Makita et al., 2011). In Libya serological survey of brucellosis carried out by El Sanousi and Omer (1985) on sera collected from 3753 cows in Benghazi using the RBPT, CFT and serum agglutination tests revealed an overall reactivity of 0.3%. Omer et al., (2000) screened samples from 2427 cattle in Eritrea, for brucella infections by the RBPT and the CFT, the highest individual seroprevalence was in dairy herds kept under the intensive husbandry system, with an individual prevalence of 8.2% and unit (herd) seroprevalence of 35.9%. However, a recent study in Eritrea was conducted by (Scacchia et al., 2013) where samples were screened with (RBPT) and the positive cases were confirmed with (CFT). A total of 2.77% of the animals tested in were positive. In Sudan El-Ansary et al., (2001) reported 5% prevalence rate in cattle in Kassala State. In Khartoum State, Ebrahim, (2013) tested 300 sera only 77 was positive by RBPT 25.7%. In Kuku Dairy Scheme Khartoum North, Sudan. The herd prevalence rate was 90%, individual animal prevalence rate was 24.9% based on C-ELISA (Angara, 2005).

### 1.14.3. Camel brucellosis

In Ethiopia, 646 camel (Camelus dromedaries) were tested by CFT 1.5% were seropositive (Warsame et al., 2012). In Egypt as reported by El-Taweel
(1999), a sero-survey study applied on camels in Cairo abattoir during 1998 showed that the incidence of brucellosis was 7.02%. In Libya Gameel et al., (1993) tested sera of 967 camels of both sexes for antibodies to brucella using the RBPT, SAT and the CFT. The prevalence of positive sera was 4.1%.

In Egypt El-Boshy et al., (2009) examined 340 dromedary camels for brucellosis using SAT and CFT. The prevalence was 7.35% by both tests. In Saudi Arabia, Radwan et al., (1995) found that the overall brucella seroprevalence was 8% in camels. In the United Arab Emirates Afzal and Sakkir (1994) detected antibodies against *Brucella abortus* in 1.5% of racing camels studied from different regions of Abu Dhabi, the RBPT and C-ELISA were used as screening and confirmatory tests, respectively. The overall seroprevalence of Brucella antibodies was 7.00% (Mohammed et al., 2013). In Jordan, study of the prevalence of camel brucellosis has been carried out in the south province of Jordan during the years 2006 and 2007 by Dawood (2008). The true prevalence of Brucella seropositive was 15.8%.

In Sudan studies on brucellosis among domestic animals in Kassala State (El-Ansary et al., 2001) who tested 64 camel sera, none were positive reactors. In Darfur States, Raga (2000) studied the disease, she failed to isolate the organism from camels, but reported 5.3% serologically. In Khartoum State (Saad, 2013) tested 415 camels and found 5.8% individual prevalence by RBPT, he found that the herd size, age of animal, mixed herd are significant as risk factors. However governate, sex, breed, herd type feeding, management type, production type, contact with other camel herd, source of new camel, milk hygiene, herd man education, awareness of brucellosis and veterinary supervision were not significant.


In Egypt the estimated annual economic losses due to brucellosis were about 60 million Egyptian pounds (AOAD, 1995). In Sudan (Angara, 2005) estimated the cost of brucellosis in Kuku dairy scheme. The total losses accounted to Sudanese Dinar (SD) 66,910,503 equivalent to US$ 267,642.
2.1. The Study Area

Jebel Aulia locality is one of the seven localities of Khartoum State. It located in the southern part of Khartoum State. The locality is bounded by White Nile State from the south, the White Nile River from west and Khartoum locality from north and east. The climate is similar to the climate of the whole State; semi-desert, dry and hot in summer (maximum temperature of 47.1°C and minimum temperature of 22.7°C). The range of rainfall is 150 mm per year.

The animal population in the locality consists of rumminant, equine and poultry. Rumminant include mainly cattle are the great percent then goat and sheep and finally camel (table 1). According to Anon (2009), the numbers of livestock holding in Jebel Aulia locality were 410 for cattle, 16 for camel, 71 for sheep and 783 for goat. Most of goats are raised in the residential areas. Table 1 below presents the number of livestock population in Khartoum State and Jebel Aulia locality.

**Table 1: Livestock population in Khartoum State and Jebel Aulia locality**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Khartoum State</th>
<th>Jebel Aulia locality</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>240,000</td>
<td>20,360</td>
<td>8.48</td>
</tr>
<tr>
<td>Camels</td>
<td>7,000</td>
<td>45</td>
<td>0.64</td>
</tr>
<tr>
<td>Sheep</td>
<td>513,000</td>
<td>9,317</td>
<td>1.82</td>
</tr>
<tr>
<td>Goats</td>
<td>624,000</td>
<td>17,819</td>
<td>2.86</td>
</tr>
</tbody>
</table>

Source: calculated by the researcher based on (2009) census.
The breeds of cattle found in Jebel Aulia are local breeds mainly Butana and crossbred between local breed and Friesian with different percent (mostly less than 75%). The dominant goats breeds are Saneen, Nubian, Shami goats and crossbred between them with different percentage. The main sheep breeds in Jebel Aulia locality are desert sheep (Ashgar, Dubassi), Hamari however it is across bred from them. The main breeds of...
camels are Bushari and Kabashi. Most of owners raising camels for the curative value of their milk and for personal use.

The study was carried out through the period from April 2012 to April 2014.

2.2. Sources of Data

2.2.1. The primary sources of data

Sample size and design and questionnaire for epidemiological and economic data surveys.

2.2.1.1. Sample size and design.

The need to use the herd as the basic statistical unit for the economic analysis, beside the lack of an appropriate sampling frame, justified the use of the multi stage cluster sample (Otte and Gumm, 1997). The number of clusters (herds) was calculated using the following formula according to Bennett et al., (1991).

$$C = \frac{P(1-P)D}{SE^2n}$$

Where C: the number of clusters to be sampled, P: the expected prevalence, D: the design effect of using cluster sample instead of simple random sample, SE: the standard error of the estimate and n: the average number of animals/holding.

According to previous prevalence the following numbers of clusters were calculated for the locality:

D = 4 (Put et al., 1988)

SE = 0.05

n= 20 (Anoon, 2009)

P for Jebel Aulia locality 51%. (Average of the six localities according to Anoon,2011)

According to the size of the herds, cattle herds were divided into three strata: small herds (<20), medium herds (21<50) and the large herds more than (50). The number of cluster selected from each stratum depended
on the stratum size. For the number of cattle selected from each cluster simple random method was adopted. Since brucellosis is a disease of sexually mature animals, only mature cows were tested. For sheep and goat 10% from the herd were sampled. The number of she camels in the locality were 20; hence all female were sampled. There was some difficulty encountered during the collection of samples. These were the difficulty in restraining some animals and that some owners refused the collection of the samples from their herd. Both acted to reduce the sample size. As a result a total of 53 herds/flocks of different species included 29 herds of cattle, 5 herds of camels, 8 herds of sheep and 11 herds of goats were selected for this study.

2.2.1.2. **Samples for serological examinations**

The skin at the site of vein puncture was swabbed with 70% alcohol then 5-10ml venous blood was withdrawn from the milk vein for cattle and jugular vein for camel, goats and sheep using disposable syringes. The syringes were placed in racks and the blood was left to stand at ambient temperature for 1 to 2 h in slanting position until the clot begins, then after that the blood was transferred to the lab in Sudan University in thermo flasks with minimal possible shaking. Blood was centrifuged before serum was separated into cryo tubes, then the serum was transferred to Veterinary Research Institute (VRI) Soba for serological testing. All sera samples were kept at -20 C before serological tests.

2.2.1.3. **Collection of epidemiological and economic data**

A questionnaire was designed to collect data required for epidemiological and economic analysis from each farm. The questionnaire was administered by the researcher and every farm owner or manager was personally interviewed. The epidemiological data included:

A-Personal data of the farm owner
b- The farm management practices
C-Herd data
d- Animal health data
E-Awareness of the owners about brucellosis

A case recording form for each positive animal was used to collect data on the age of the cow, breed, number of births, number of abortions, cases of retained placentas, previous mastitis and finally any other infections. The questionnaire also included data required for the economic analysis those were:

- Milk yield.
- Price of milk.
- Price of replacement heifers.

2.2.2. The secondary sources of data


2.3. Serological Tests

The main serological test used in this study for diagnosis of brucellosis is the Rose Bengal Plate Test (RBPT), which has very high (>99%) sensitivity but low specificity (Barroso et al., 2002). The positive results were confirmed by C-ELISA and only animals positive on both RBPT and C-ELISA were classified Brucella seropositive.

2.3.1. Rose Bengal Plate test (RBPT)

All serum samples were screened by the RBPT for the presence of antibodies against Brucella antigens. The antigen and the serum samples were removed from the refrigerator to room temperature and shaken properly before use. Equal quantity of serum sample and (RBPT) antigen (30μl) were taken on an enamel plate, mixed thoroughly with metal stick. The plate was then shaken on a rocker for 4 min. The result was read immediately after 4 min. Any degree of agglutination was considered as positive reaction. Agglutination appeared as weak positive, positive, strong positive or very strong positive (Alton et al., 1988).

2.3.2. Competitive enzyme linked immuno-sorbent assay (C-ELISA)
This test used as a confirmatory test to eliminate any positive reaction in the (RBPT) due to vaccination or cross reaction. C-ELISA kit obtain by (Veterinary Laboratory Agency, New Haw, Addlestone, Surrey KT 15 3NB United Kingdom. Version 2.0, June 2012) COMPELISA.

2.3.2.1. Kit contents

The kit was refrigerated immediately on arrival and the conjugate stored at -20°C. The content were:

1- Plates: Plates pre-coated with *Brucella melitensis*, Lipo polysaccharide (LPS) antigen and Lid.

2- Diluting buffer: Tablets of phosphate buffered saline (PBS), phenol red indicator and Tween 20.

3- Wash solution: Na2HPO4 and Tween 20.

4- Conjugate: As supplied (store at -20°C).

5- Chromogen: O-Phenylene diamine (OPD) tablets (Toxic).

6- Substrate: Urea hydrogen peroxide tablets (irritant).

7- Stopping solution: Citric acid (irritant).

8- Controls: Positive serum and negative serum.

2.3.2.2. Equipment required

1- Microtitre plate reader with 450 nm filter. It is not essential; the result can be performed visually.

2- Single and multichannel variable volume pipettes.

3- Disposable tips for the above.

4- Reagent troughs for multichannel pipetting.

5- 10 L container for wash fluid.

6- 4 ±3°C refrigerator.

7- Rotary shaker, capable 160 Revs/Min (or a 37 ± 3°C incubator).(by adapting the method their use is not essential).

8- Microtitre plate shaker.

9- Sterile distilled or de ionized water.

10- Bottles tubes and beakers for storage of sera and reagents.
11- Absorbent paper towels.
12- Freezer for storage of conjugate.

2.3.2.3. Reagent preparation

2.3.2.3.1. The diluting buffer

The diluting buffer was prepared by adding 5 tablets of PBS, 0.5 ml phenol red indicator and 250 μl of Tween 20 to 500 ml distilled water. The pH was kept between 7.2 and 7.6 by testing the buffer with phenol red which turns yellow below pH 7.2 and violet above pH 7.6. The buffer was stored at 4 °C and used during one month period.

2.3.2.3.2. The wash solution

The wash solution was prepared by adding the contents of the ampoule of Na2HPO4 (0.14 g) and 1 ml of Tween 20 to 10 L of distilled water. The solution was stored at room temperature and used during one month period.

2.3.2.3.3. The conjugate

The conjugate was prepared by adding 1 ml of the content of the conjugate ampoule to 11 ml of diluting buffer to give 12 ml of the conjugate. Once the conjugate had been prepared according to instructions on the ampoule, it was used immediately.

2.3.2.3.4. The stopping solution

The stopping solution was prepared by diluting the contents of the ampoule of citric acid (2 ml) with 38 ml of distilled water and stored at 4 °C and used during one month period.

2.3.2.3.5. The controls

Each of the positive and negative control samples included in the kit were reconstituted with 1 ml sterile distilled water. They were allowed to stand until an even suspension was obtained. The entire contents were completely suspended before use. Store at 4 °C.

2.3.2.4. Method
1. The conjugate solution was prepared and diluted to working strength with diluting buffer according to instructions on the ampoule label.
2. 20 μl of each test serum were added per each well. Columns 11 and 12 were left for controls.
3. 20μl of the negative control was added to the wells, A12, B11, B12, C11 and C12.
4. 20 μl of the positive control were added to the wells F11, F12, G11, G12, H11 and H12.
5. The remaining wells have no serum added and act as the conjugate controls.
6. 100 μl of the prepared conjugate solution were dispensed immediately into all wells. This gave a final serum dilution of 1/6.
7. The plate was then vigorously shaken (on the microtitre plate shaker) for 2 min in order to mix the serum and conjugate solution. The plate was covered with the lid and incubated at room temperature for 30 min on a rotary shaker, at 160 revs/min.
8. The contents of the plate were shaken out and rinsed 5 times with washing solution and then thoroughly dried by tapping on absorbent paper towel.
9. The microplate reader was switched on and allows the unit to stabilize for 10 min.
10. The substrate and chromogen solutions were prepared immediately before use by dissolving one tablet of urea hydrogen peroxide in 12 ml of distilled water. When dissolved, the OPD tablet was added and mixed thoroughly using a magnetic stirrer. Then 100 μl was added to all wells.
11. The plate was left at room temperature for 15 minutes.
12. The reaction was slowed by adding 100 μl of stopping solution to all wells.
13. The condensation in the bottom of the plate was remove with absorbent paper towel and the plate was read at 450 nm.

The colour of the wells was compared with the negative (coloured) control wells and the positive (clear) control. A positive/negative cut-off was
calculated as 60% of the mean of the optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value was regarded as being positive.

2.4. Data Analysis

2.4.1. Prevalence rates

Data was stored in the Microsoft excel spread sheet and the herd prevalence and individual prevalence were calculated for whole ruminants and for each animal species.

2.4.2. Risk factors analysis

Data on risk factors obtained from the study questionnaire were stored in a computer data base and statistical analysis was performed using statistical package for social science (SPSS) version 16.0 for windows. First frequency analysis was performed to know the distribution of potential risk factors according to the seropositive animal and their percent. Then the univariable analysis cross tabulation was performed to test the association between each brucellosis seropositive status and potential risk factors (chi square). Only risk factors with significant value \((p\)-value\) \(<0.25\) were considered to be significant.

The multivariable logistic regression was used to analyze the associations of various risk factors with the seroprevalence of the disease using Wald test. Only variables with \(P\)-values \(<0.25\) in univariable analysis were tested in the logistic regression model, except the breed of animals (only cattle breed was tabulated). Only risk factors with \(P\)-values \(<0.05\) were considered to be significant.

2.4.3 Analysis of the economic data

From the known methods of economic impact assessment of animal disease partial budgeting according to Morris (1999) was considered to be the best method to evaluate the economic impact of an endemic disease.

2.4.3.1. Parameters used and their sources
1. The total number of mature cows in the locality = 12,684 heads (Anon, 2009).

2. The total number of mature cows in the herds studied = 739 heads (the field survey).

3. The total number of mature cows sampled = 207 heads (the field survey).

The following parameters were estimated:

4. Sero prevalence rate.
5. Abortion rate.
6. Repeat breading rate.
7. Reduction in milk production by 20% for aborted and 10% for non-aborted cows (Zinsstag et al, 2005).
9. The average price of milk/L.
10. Price of female calf at weaning weight
11. Price of male calf at weaning weight
12. Cost of repeat breeding due to brucellosis.

2.4.3.2. Calculation of economic loss of bovine brucellosis

2.4.3.2.1 Calculation of economic loss of bovine brucellosis in the selected sample.

Total Loss due to bovine brucellosis in the sample= losses due to reduction in milk production + loss due to infertility (Losses due to abortion + Losses due to repeat breeding).

1. **Losses due to reduction in milk production**

   Total quantity of milk lost= Quantity of milk loss of seropositive aborted animals+ quantity of milk loss of seropositive non- aborted animals.

   Quantity of milk loss of seropositive aborted animals= (Number of aborted seropositive animals x average annual milk yield x 20%). quantity of milk
loss of seropositive non-aborted animals = (Number of non-aborted seropositive animals x average annual milk yield x 10%).

Value of milk lost = Total quantity of milk lost x price of milk ….. (1)

2. Losses due to loss of aborted foeti

Number of aborted foeti in the sample = Number of aborted seropositive cows.

We supposed that 50% of the aborted foeti were female and the rest 50% were male.

Value of lost foeti = Number of aborted female foeti x price of female calf at weaning + Number of aborted male foeti x price of male at weaning………………….. (2)

3. Loss due to repeat breeding

Number of repeat breeding cows = repeat breeding rate x seropositive animals.

The financial losses due to repeat breeding = Number of repeat breeding cows x Cost of repeat breeding due to brucellosis/cow…………….. (3)

Total Loss due to bovine brucellosis in the sample = Equation 1 + 2 + 3

2.4.3.2.2. Calculation of economic loss of bovine brucellosis in the herd studied.

Total Loss due to bovine brucellosis in the herd studied = Total Loss due to bovine brucellosis in the sample x number of mature cows in the herd sampled/ number of mature cows in the sample.

2.4.3.2.3. Calculation of economic loss of bovine brucellosis in the whole locality

Total Loss due to bovine brucellosis in the locality = Total Loss due to bovine brucellosis in the sample x number of mature cows in the locality/ number of mature cows in the sample.
CHAPTER THREE

RESULTS

3.1. The Prevalence Rates of Ruminant’s Brucellosis in Jebel Aulia Locality.

The serological tests revealed that out of the 53 herd tested, 34 showed at least one positive animal by RBPT, accordingly the overall herd prevalence was 64% (34/53). The prevalence rate for cattle was 90% ((26/29), for camels was 20% (1/5), for goats was 36% (4/11) and for sheep was 38% (3/8). The confirmatory test using competitive enzyme linked immuno sorbent assay (C-ELISA) indicated that the overall herd reactivity was 49% (26/53). The cattle herd reactivity was 76% ((22/29) for cattle, 20% (1/5) for camels, 18% (2/11) for goats and 13% (1/8) for sheep (Table 2).

Table 2: The herd Prevalence rates of ruminant’s brucellosis in Jebel Aulia locality.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>RBPT</th>
<th>C-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of herds examined</td>
<td>+ve herds</td>
</tr>
<tr>
<td>Cattle</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Camels</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Goats</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Sheep</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>34</td>
</tr>
</tbody>
</table>

For all animals the positive serum samples by RBPT were 21% (84/393). The individual animal prevalence was 35% (72/207) for cattle, 5% (1/20) for camels, 9% (7/82) for goats and 5% (4/84) for sheep. The (C-ELISA) test revealed 11% (43/393) overall individual animal prevalence, 19%
(39/207) for cattle, 5% (1/20) for camels, 4% (3/82) for goats and 1% (1/84) for sheep (Table 3).

**Table 3: The Individual Animal Prevalence rate of brucellosis in ruminants in Jebel Aulia locality.**

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Diagnostic test</th>
<th>Number of samples examined</th>
<th>+ve samples</th>
<th>Prevalence rate%</th>
<th>Number of samples examined</th>
<th>+ve samples</th>
<th>Prevalence rate%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>RBPT</td>
<td>207</td>
<td>72</td>
<td>35%</td>
<td>207</td>
<td>39</td>
<td>19%</td>
</tr>
<tr>
<td>Camels</td>
<td></td>
<td>20</td>
<td>1</td>
<td>5%</td>
<td>20</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>Goats</td>
<td></td>
<td>82</td>
<td>7</td>
<td>9%</td>
<td>82</td>
<td>3</td>
<td>4%</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td>84</td>
<td>4</td>
<td>5%</td>
<td>84</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>393</td>
<td>84</td>
<td>21%</td>
<td>393</td>
<td>43</td>
<td>11%</td>
</tr>
</tbody>
</table>

**Figure 2 : Hygroma in fore limb**

Abortion rate = Number of aborted cows in the sample/Total cows studied = 0.068

Repeat breading rate = 0.15 (infertility rate) - abortion rate=0.08 (Zinsstag et al., 2005)
3.2. Potential Risk Factors Associated with Brucellosis in Jebel Aulia Locality.

3.2.1. Frequency and distribution of potential risk factors associated with brucellosis

The result of frequency distribution indicates that 77% of the farms of Jebel Aulia locality were located in Jabel Aolia unit (Appendix 3).

Although 43% of the owners were illiterate, 62% of them used to vaccinate their animals by the package of vaccines described by the veterinary authorities (anthrax vaccine, B.Q. vaccine, H.S. vaccine and C.B.P.P. vaccine) for cattle and the first three vaccines for camel, goat and sheep. Yet, only 4% of the herds were vaccinated against Brucella. The confirmed herd seroprevalence of brucellosis in the locality was 49%. This was clinically manifested by the abortion in about 38% of the herd population. Yet 72% of the animal owners have no knowledge of the cause of these abortions.

According to the breeding practice as a risk factor, many owners depend on natural breeding and about 91% of them have their own bull.

The association between Brucella prevalence and the management risk factor showed that about 60% of the animal reared are purchased from the local market and kept in multi-species pattern in 55% of the farms. Feeding of these animals depend mainly on free grazing in about 45% to reduce the cost of feeding. About 62% of these animals are fed separately 89% of the animal population have their own source of water.
Figure 3: A multi species herd sharing drinking water container

3.2.2. Univariable analysis and chi square test for risk factors.

In (table 4), the chi-square univariable analysis was performed to test the relationship between prevalence and potential risk factor. The chi-square univariable analysis revealed nine variables with $p$-value $\leq 0.25$ were statistically significant. The test revealed there was significant association between the rate of infection and the unit ($p$-value=0.041), owner education ($p$-value = 0.120), type of herd ($p$-value = 0.020), dealing with sick animal ($p$-value = 0.181), presence of abortion cases ($p$-value = 0.018), knowledge of the owner about the cause of abortion ($p$-value = 0.041), having their own bull for breeding ($p$-value = 0.172), feeding and watering practices ($p$-value = 0.006), the source of water ($p$-value = 0.092).

Table 4. Chi square test for the association brucellosis prevalence and risk factors
<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. Positive</th>
<th>( \chi^2 )</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jebel Aulia</td>
<td>17</td>
<td>4.178</td>
<td>1</td>
<td>0.041</td>
</tr>
<tr>
<td>Elazhry</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owner education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>15</td>
<td>4.247</td>
<td>2</td>
<td>0.120</td>
</tr>
<tr>
<td>Rather good</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well educated</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of the herd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One species</td>
<td>16</td>
<td>5.44</td>
<td>1</td>
<td>0.020</td>
</tr>
<tr>
<td>Multi-species</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dealing with sick animal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have veterinarian</td>
<td>23</td>
<td>1.791</td>
<td>1</td>
<td>0.181</td>
</tr>
<tr>
<td>No veterinary care</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No abortion cases</td>
<td>12</td>
<td>5.638</td>
<td>1</td>
<td>0.018</td>
</tr>
<tr>
<td>Have abortion cases</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knowing the cause of abortion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well knowledge</td>
<td>4</td>
<td>4.197</td>
<td>1</td>
<td>0.041</td>
</tr>
<tr>
<td>Bad knowledge</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have a bull</td>
<td>25</td>
<td>1.865</td>
<td>1</td>
<td>0.172</td>
</tr>
<tr>
<td>Borrow bull</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding and watering animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separate</td>
<td>21</td>
<td>7.483</td>
<td>1</td>
<td>0.006</td>
</tr>
<tr>
<td>Together with other species</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have source</td>
<td>25</td>
<td>2.840</td>
<td>1</td>
<td>0.092</td>
</tr>
<tr>
<td>Common canal</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3.2.3 Logistic regression for testing the association between brucellosis prevalence and the risk factors

In (table 5) the nine factors with \( p \)-value \( \leq 0.25 \)in the univariable analysis were subjected to multivariate logistic regression using Wald test. The test revealed that only presence of abortion cases (OR.001, CI.00-.247, \( p \)-
value.014) and the source of water (OR1.51, CI 2.949-7.745E5, p-value.021) were significant (P<0.05).

Table 5. Summary of Multivariate analysis for potential risk factors of animal brucellosis examined in Jebel Aulia using Logistic Regression (Wald test).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. positive herds</th>
<th>Exp(B)</th>
<th>95% C.I for Exp(B)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No abortion case</td>
<td>12</td>
<td>.001</td>
<td>.00-.247</td>
<td>0.014</td>
</tr>
<tr>
<td>Have abortion case</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have source</td>
<td>25</td>
<td>1.511E3</td>
<td>2.949-7.745E5</td>
<td>0.021</td>
</tr>
<tr>
<td>Common canal</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3. Estimation of the Financial Loss due to Bovine Brucellosis.

3.3.1. Estimation of the financial loss due to bovine brucellosis in the selected sample.

The financial loss due to reduction of milk production: 1

1. Losses due to reduction of milk production =

Quantity of milk loss of seropositive aborted animals (Number of aborted seropositive animals x average annual milk yield/year x 20%) + quantity of milk loss of seropositive non-aborted animals (Number of non-aborted seropositive animals x average annual milk yield x 10%).

Average annual milk yield= 2,614 L (Medani,1996).

The average price of milk/L = SDG 3 (field survey).

= 14 \times 2,614 \text{ L} \times 20\% + 22 \times 2,614 \text{ L} \times 10\% 
7,319,2 + 5,750,8 = 13,070 \text{ L}

Value of milk lost = Total quantity of milk lost x price of milk…… (1)

= 13,070 \times 3 = 39,21 SDG.
2. **Losses due to loss of aborted foeti:**

Number of aborted foeti in the sample = Number of aborted seropositive cows.

We supposed that 50% of the aborted foeti were female and the rest 50% were male.

Price of female calf at weaning weight = SDG1000 (field survey).

Price of male calf at weaning weight = SDG800 (field survey).

Value of lost foeti = Number of aborted female foeti x price of female calf at weaning + Number of aborted male foeti x price of male at weaning........................ (2)

\[ 7 \times 1000 + 7 \times 800 = 12,600 \text{ SDG} \]

3. **Loss due to repeat breeding:**

Number of repeat breeding cows = repeat breeding rate x seropositive animals = 0.08 x 39 = 3.12 cows = 3 cow

Cost of repeat breeding due to brucellosis = SDG 1.24/cow (adapted from Angara and Elfadil, 2014).

The financial losses due to repeat breeding = Number of repeat breeding cows x Cost of repeat breeding due to brucellosis/cow.............. (3) = 3 x 1.24 = 3.72 SDG

Total Loss due to bovine brucellosis in the sample = Equation 1 + 2 + 3

\[ 39,21 + 12,600 + 3.72 = 55,53 \text{ SDG} \]

Total loss in the sample studied = 55,530 SDG
3.3.2. Estimation of the financial loss due to bovine brucellosis in the herds studied.

Total loss in the herd studied = 55,530 / 207 x 739 = 198,245 SDG

3.3.3. Estimation of the financial loss due to bovine brucellosis in the whole locality.

Total loss in whole locality = 55,530 / 207 x 12,684 = 3,402,620 SDG
*4.6 (SDG) per US dollar in (2012) in the study.(Anoon 2014).

Table (6): The total economic loss due to brucellosis in Jebel Aulia

<table>
<thead>
<tr>
<th>Item</th>
<th>SDG</th>
<th>US$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss in milk</td>
<td>39,210</td>
<td>8,524</td>
</tr>
<tr>
<td>Loss in calve</td>
<td>12,600</td>
<td>2,739</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>-----</td>
</tr>
<tr>
<td>Loss in fertility</td>
<td>3,120</td>
<td>678</td>
</tr>
<tr>
<td>Total loss in the sample</td>
<td>55,530</td>
<td>12,072</td>
</tr>
<tr>
<td>Total loss in the herds sample</td>
<td>198,245</td>
<td>34,097</td>
</tr>
<tr>
<td>Total in the locality</td>
<td>3,402,620</td>
<td>739,700</td>
</tr>
</tbody>
</table>
CHAPTER FOUR
DISCUSSION

The RBPT is widely used in Sudan in screening brucellosis for regulatory control and for export requirements. Although the test is very sensitive and suitable for screening herds for brucellosis, it can give false positive results due to vaccination *Brucella abortus* strain 19 vaccine or due to cross reaction with other bacteria (OIE,2004). This fact justifies the use of C-ELISA as a confirmatory test. This has been verified by the result obtained in this study (tables 2 and 3) where many of the false positive of the RBPT were ruled out using C-ELISA.

The prevalence of brucellosis was found to be high in Jebel Aulia locality despite climatic conditions of the State (persistence of the sun light at the most hours of the day, dry desert weather and low humidity) which may not favor survival of Brucella organisms for long periods. Among ruminant animals tested cattle were found to be mostly affected by the disease. The high prevalence rate in cattle in this study is attributed to the management practice where cattle are kept overcrowded and reared in open system in which animals of different ages; aborted and pregnant ones; males and females are housed together in high stocking density. This in addition to the fact that infected animals shed the organism in the after birth discharges of the aborted or normally delivering animals. More over in the traditional sector, infected animals are usually kept for breeding despite the fact that congenital infection is a major epidemiological means of spread of the disease as it is well known that as high as 20% of calves born by infected heifers could be found persistently infected with *Brucella* (Nielsen and Duncan, 1990).

Camels came after cattle in harboring the infection. All camel herds in the locality were tested because they are few and they can easily be restrained.
On the other hand, sheep show low prevalence rate of brucellosis which may be attributed to the fact that sheep kept were mainly males brought from range lands for marketing purposes, more over they are usually raised in extensive system whereas the levels of Brucella infections tend to be relatively high in intensive systems (Anonymous, 1986).

The individual seroprevalence of bovine brucellosis in Jebel Aulia determined in this study was 35% using RBPT. As a comparison with other related studies, very low prevalence (3.66%) was reported in Iran based on Akbarmehr and Ghiyamirad (2011). According to Makita et al., (2011) the individual prevalence of bovine brucellosis in Kampala was 5.0%, using (C-ELISA). In Libya El Sanousi and Omer (1985) reported over all reactivity of 0.3%. Moreover, lower cattle prevalence (25.7%) was reported in Khartoum State by Ebrahim (2013) using RBPT. Also a lower rate (5%) was reported in Kassala State by El-Ansary et al., (2001).

The incidence of bovine brucellosis seems to be higher in intensive farming rather than extensive ones. Higher result (24.9%) was obtained by Angara et al., (2009) in Kuku Dairy Scheme Khartoum North, and Sudan based on c-Elisa. They also reported higher herd prevalence rate (90%) using the same test. Higher rates in Kuku Scheme were due to high foreign blood breed kept beside the presence of the animal in close contact with indigenous breeds.

The prevalence of Brucella antibodies in the sera of camels obtained in this study was (5%). El-Taweel (1999) reported a bit higher prevalence of 7.02 % of camel sera in Egypt, as well higher rates of (7.35%), was reported by El-Boshy et al., (2009). Higher rates of 8%, 7.00% and 15.8%, were also reported by Radwan et al., (1995), Mohammed et al., (2013) and Dawood (2008) in Saudi Arabia, Abu Dhabi and Jordan respectively. In Darfur and Khartoum States, Raga (2000) and Saad (2013) reported slightly higher rates of camel brucellosis of 5.3% and 5.8% respectively. Lower rates of camel brucellosis were reported in Libya (4.1%), United Arab Emirates (1.5%).
Ethiopia (1.5%) and Kassala State, Sudan (0%) by Gameel et al., (1993), Afzal and Sakkir (1994), Warsame et al., (2012) and El-Ansary et al., (2001) respectively.

Regarding Small ruminant brucellosis, the infection rate in small ruminant reported in the current study which is 36% is far lower than that reported in the Palestinian Authority (72.9%) by Shuaibi (1999). The very high rate of infection in small ruminant reported by Shuaibi (1999) was due to the fact the herds tested were selected purposely for being suspected to be infected due to abortions cases noted between them or the presence of human cases, whereas herd tested in the current study were selected randomly.

Goats’ seroprevalence in Jebel Aulia obtained by RBPT was 9% and the herd prevalence was 36%. These results were higher than the results obtained by Omer et al., (2000) in eastern part of Eritrea he got 8.3% as individual prevalence. The herd prevalence rate was 33.3%. However, higher individual prevalence rate of 14.3% and higher unit prevalence of 56.3% were reported by same author in western part of Eritrea. In Yemen (Hosie et al., 1985) reported the prevalence among goats was lower 0.4%. El-Ansary et al. (2001) in Kassala State, Sudan reported similar individual prevalence 4% in goats. A lower prevalence of 2.0% in goat in Khartoum was reported by Magzub (2001). Omer et al., (2007) in Kassala eastern Sudan during 2004-2006. Reported lower prevalence the result was that: prevalence rate was 0.2%, 0.6% respectively while in 2006 it was 5.6% in goats.

The seroprevalence of ovine brucellosis determined in this study was 5% while the herd prevalence was 38%. The individual animal prevalence was found to be higher than the rate of 1.4% obtained by Omer et al., (2000) in Eritrea. This also true for the herd prevalence where it is 16.7% in Eritrea. Lower sheep prevalence (0.6%) was reported in Yemen by Hosie et al., (1985).

The current result was higher than (0%) rate in sheep reported by Cadmus et al., (2006) in Nigeria and the rate of 1% in Kassala State, Sudan
reported by El-Ansary et al., (2001). Ali (2013) reported lower prevalence of 2.5% in North Kordofan. Omer et al., (1989 to 1990) reported lower prevalence in (Alkdrui) quarantine, Khartoum state and (Portsudan) quarantine, Red sea State the prevalence rate of sheep brucellosis was 0.01%. As well Omer et al., (2007) reported low prevalence of ovine brucellosis in Kassala eastern Sudan during 2004-2006, the result was that the prevalence rate was 0.1%, 0.4% respectively however in 2006 the prevalence was 2.1%. Musa (2005) reported higher rate (3.3%) of sheep brucellosis in Darfur States. As well higher results of 4.1% was reported by Omran (2011) in Sinnar.

The significant association between the rate of infection and the owner education based on univariate analysis reflected owners’ ignorance about the importance of vaccinating their animals against brucellosis. Although 62% of them vaccinate their animal against common infectious diseases, yet only 4% of the herds were vaccinated against *Brucella*. This comes in agreement with Teshale et al., (2006) who attributed the high prevalence of Brucella antibodies and widespread nature of Brucella infection 9.7% in small ruminants in Afar, Somali region to the absence of Brucella vaccination.

The findings of the univariate analysis in the current study which revealed that keeping multi species herds and sharing the same source of water is a potential risk factors for brucellosis. This is similar to studies of (Al-Majali et al., 2009; Muma et al., 2007) in which they found the practice of mixing of cattle, either through grazing or sharing of watering points is an important risk factor for brucellosis. Also Megersa et al., (2011) reported that keeping more than two animal species at household level was found to be a risk factor for cattle and camel seropositivity to Brucella infection when compared to those animals from households that keep only two animal species. This may suggest a possibility of cross species transmission of Brucella infection under such mixed herding. Surprisingly dealing with sick animal as a risk factor for brucellosis, 88.5% of the owners indicated that they have access to veterinary services. This may be attributed to the fact that most
of the owners consult individuals referred to as technician who have no adequate knowledge on veterinary service.

The association between Brucella seropositivity and abortion comes in agreement with Matope et al., (2011) and Aulakh et al., (2008). Using special bull for breeding appeared as a risk factor for brucellosis. Although that 91% of the holdings have their own bull and the transmission by natural insemination is very weak, this association may be attributed to the high stocking density and the mechanical transmission. The source of water appeared as a risk factor for brucellosis in this study. Although that 89% of the farms have their own source of water, yet 25 of herds were found to be seropositive. This may be due to the bad hygienic management within the farm where different ages and multispecies share the same container.

Logistic regression model revealed that only presence of abortion case and the source of water were significant (P<0.05). As a fact mentioned before, brucellosis causes a decrease in reproductive efficacy and an increase abortion rate (Rijpens, et al., 1996). So in addition to bad sanitary measures in farms, abortion play major role in spreading the infection. This result is similar to the result of Tesfaye et al., (2011) in investigation of bovine brucellosis and associated risk factors in Addis Ababa dairy farms. The study revealed 4.4% abortion was associated with Brucella antibodies (P<0.05). Islam et al., (2010) and Rahman et al., (2011) also found a significant association between abortion cases and occurrence of brucellosis (P < 0.01) in Bangladesh. Similar result also were observed by Berhe et al., (2007) when investigating 26 herds in Tigray Region of Ethiopia and found that seropositivity to brucellosis had statistically significant association with history of previous abortions and stillbirths.

The source of water as a significant risk factor in multivariate analysis comes in agreement with the studies on risk factor of brucellosis in Khartoum State by Ishag (2013) and Saad (2013) in which they found
source of feeder and water source were significant in sheep and camels respectively.

Although Sudan was proved to be endemic with brucellosis, few studies estimated the economic impact of the disease. The current study considered the financial losses in dairy sectors namely the loss in cattle dairy farms because cattle were the most important source of milk, reservoir and sufferer of brucellosis beside their role in income generation and food security.

According to the result the bulk loss was due to milk loss and this is due to Brucella effect on milk production and the majority of the farms are for dairy production, the low percent of abortion 0.07 in this study indicates the disease is endemic and not recent infection that usually result in a storm of abortion. The repeat breeding rate of 0.08 was estimated based on Zinsstag et al., (2005). This rate was used to estimate the economic loss following Angara and Elfdil (2014). The result obtained indicated that repeat breeding due to Brucella constitutes a minor cause of repeat breeding in Jabel Aolia locality, other unidentified causes exists, farmers do not managed investigate on these causes in their herds, instead infertile cows are kept without treatment or sold.

Angara, (2005) estimated the cost of brucellosis in Kuku dairy scheme where the total losses accounted to SD 66,910,503 equivalent to US$ 267,642. The total losses in this study accounted to 3,402,620 SDG equivalent to 739,700US$. The difference in the cost may be attributed to the difference in the size of the animal population studied.

There is no sufficient, analysed information on the economic importance of brucellosis in most African countries. The economic loss resulting from bovine brucellosis in Nigeria in 1979 was estimated at $223.2 million (Esuruoso, 1977). Worldwide The U.S. suffers from the same problems that face the other countries; the yearly cost of Brucellosis in the United States was $30 million, in Brazil the disease causes a 50% decrease in calf production which is much higher than that reported in this study.
Conclusion

Bovine brucellosis in Jabel Aolia locality is higher than other species. The number of camel in the locality are very few so the prevalence of camel in this study cannot give a real idea however, it gives estimation about presence of brucellosis. The prevalence in sheep and goat in the locality do not differ largely from the prevalence of brucellosis in other localities. The study proved that brucellosis causes financial losses in animal (dairy and meat production) sectors.

Recommendations

The study recommends:

1. A periodic testing for brucellosis should be introduced.
2. More efforts should be directed towards implementing a proper control program for brucellosis.
3. More animal health biosecurity need to be improved
4. There is a need to raise the awareness of the producers towards the disease and its control measure.
5. More investigation on infertility problems need to be carried
6. Movements of animals should be controlled by appropriate legislation and regulations.
REFERENCES


Bendixin H.C. and Blood E. (1947). Investigation on brucellosis in the bovine male with special regard to the spread of the disease by artificial insemination. *British veterinary journal.(103).*


Makita K.;  Fèvre M.E.; Waiswa C.; Eisler C. M.; Thrusfield M. and Welburn C,S .(2011) Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, *Uganda BMC Veterinary Research* 7:60.


Appendix 1

Questionnaire sheet for animal Brucellosis survey in Khartoum State, Sudan 2012

Date: .................... Serial No:……… …………………...

1. District

1- Khartoum
2- Omdurman
3- Khartoum North

2. Locality :

1. Jebel Aulia
2. Khartoum
3. Umbeda
4. Karari
5. Abu Saed
6. Bahri
7. Sharq Elneil

3. Administrative Unit.................................

4. Farm Location Latitude: ......................... Longitude...........

5. Personal data of the farm owner. :

1- Status of Respondent: (i) Owner (ii) Worker (iii) Farm Manager

Name (farm owner): ............................................................

Address:...........................................................................

2- Sex: (i) Female. (ii) Male.

3- Age: .........................

4- Education:

(i) Illiterate (ii) Khalwa (iii) Primary` (iv) Secondary (v) High

Secondary (vi) University (vii) Postgraduate.
6. Herd data:

1- Who long do you, operate this farm?

2- Type of the herd:
   (i) One Species  (ii) Multi-species (mixed )

3- Type of animals:
   (i) Cattle only  (ii) Cattle & goats (iii) cattle & sheep (iv) cattle & camels

4- Number of animals rose:
   (i) Cattle.......  (ii) Goat....  (iii) Sheep......  (iv )Camels ......

5- Breed of animal: (i) Cross (ii) local (iii) Pure foreign

6. Breed source) :i) imported (ii) local market (iii) bred within the farm.

7. Foreign blood % (if known): (i) 50(ii) 62.5(iii) 70(iv) more than 70

8. Herd structure:

<table>
<thead>
<tr>
<th>Age</th>
<th>Less than one year</th>
<th>1-3 y ears</th>
<th>More than 3 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Cross</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foreign</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7. Farm Labour

<table>
<thead>
<tr>
<th>occupation</th>
<th>Number of Name of worker</th>
<th>Name of worker(s)</th>
<th>Wage per month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Animal Health Data:
1. How do you deal with sick animals?

(i) Have a recruited veterinarian or veterinary technician

(ii) Call a veterinarian or veterinary technician

(iii) Consult veterinary service center.

(iv) Treat them myself.

2. Do you vaccinate your animals?

(i) Yes

(ii) No

3. If yes, what diseases do you vaccinate your animals for?

(i) Brucella

(ii) B.Q.

(iii) CCPP

(iv) H.S

(V) Anthrax

(vi) CBPP

4. Do you have abortion cases in your farm?

(i) Yes

(ii) No

5. If yes, how does it occur?

(i) Repeatedly

(ii) Sometimes

6. What do you do for an animal that aborted?

i. Nothing

ii. Treatment with antibiotic if yes type of antibiotic & cost

iii. Call-/consult a veterinarian & cost

7. Do you have stillbirth cases in your farm during the last year?

(i) Yes

(ii) No

8. Do you know the causes of it (abortion)?

(i) Yes

(ii) No
9-If yes, what are they?

1. ........................................... 3- ...........................................
2. ........................................... 4- .................................

10-Do you know about brucellosis?

(i) Yes
(ii) No

11-If yes what do you know about brucellosis?

........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

12- How do you deal with animals that proved to have brucellosis?

(i) Sell them for slaughtering.
(ii) Sell them to other farmer.
(iii) Treat them.
(iv) Keep them within the herd without treatment
(V) Keep them in a separate place:

9. **Herd management data:**

1- How do you keep your herd?

(i) Mixed p
(ii) Separated according to age
(iii) Separated according to age and sex

2-Do you have special barn for calving cages?

(i) Yes (ii) No

3- What type of breeding do you adopt?

(i) Natural insemination .
(ii) Artificial insemination.

4- In case of natural insemination, do you
(i) own 'a bull
(ii) Borrow one from other farms.

5- How do you feed and water your animals?
(i) In separate' containers
(ii) Common container

6- What is the source of the green fodder you provide to your herd?
(i) Grazing land.
(ii) From my farm’. 
(iii) Buy it from other farm.
(iv) Buy it from market.

7- What is the source of the concentrate feed you provide to your herd?
(i) Prepared within the farm« ?
(ii) Readymade concentrate.

8- What is the source of water that you provide to your herd?
(i) From irrigation canal’.
(ii) Wells
(iii) Tap water
(iv) Transported by donkeys.

13. The Prices of farm produces.


II. Price of male kid at weaning  IV. Average milk production per day.
Appendix 2
Case recording form for brucellosis seropositive animals.

Farm Number
Sample Number
Animal ID
Age of the animal
Animal breed
Number of birth
Number of abortion
Cases of retained placentas
Previous mastitis infection
Other infection
RBT result
C-Elisa result

Appendix 3
Frequency distribution of potential risk factors examined for brucellosis in 53 herds.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>distribution</th>
<th>percent</th>
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<tr>
<td><strong>Unit</strong></td>
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</tr>
<tr>
<td>Jebel Aulia</td>
<td>41</td>
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<td>Elazhry</td>
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<tr>
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</tr>
<tr>
<td>Well educated</td>
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<td>35.8</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Total</td>
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<td>100.0</td>
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<td>Have veterinarian</td>
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<td>No veterinary care</td>
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<tr>
<td>Total</td>
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<tr>
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<td>Repeatedly</td>
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<tr>
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</tr>
<tr>
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<tr>
<td>Bad knowledge</td>
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<tr>
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## Appendix 4

Univariable analyses for factor have more than 25 for $p$-value

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<tr>
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**Appendix 5**

**Multivariable analyses for factors have more than 0.05 p-value**

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<th>Risk factor</th>
<th>No. positive herds</th>
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<th>95% C.I for Exp(B)</th>
<th>P-value</th>
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<tr>
<td><strong>Unit</strong></td>
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<td><strong>Feeding and watering animals</strong></td>
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