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 mammles, 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 (Mcmahan, 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 cellularlly 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 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 present 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). RBPTis 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 it consider 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 mono clonal 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 (Nielson 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 a symptomatic 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 rule in spreading the infection However in artificial insemination because the infected semen is ejaculated in to 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. *Brucella*e 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 erythirtol 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 by30% 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. Related Studies on Prevalence, Risk Factors and Financial Loss of Brucellosis.

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% and3.8% 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 (Alkdru) 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

goatsin 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, SATand 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 sero-prevalence 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.

1.14.4. Financial loss of brucellosis.

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.

CHAPTER TWO

MATERIALS AND METHODS

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

Animal	Khartoum State	Jebel Aulia locality	percent
Cattle	240,000	20,360	8.48
Camels	7,000	45	0.64
Sheep	513,000	9,317	1.82
Goats	624,000	17,819	2.86

Source: calculated by the researcher based on (2009) census.

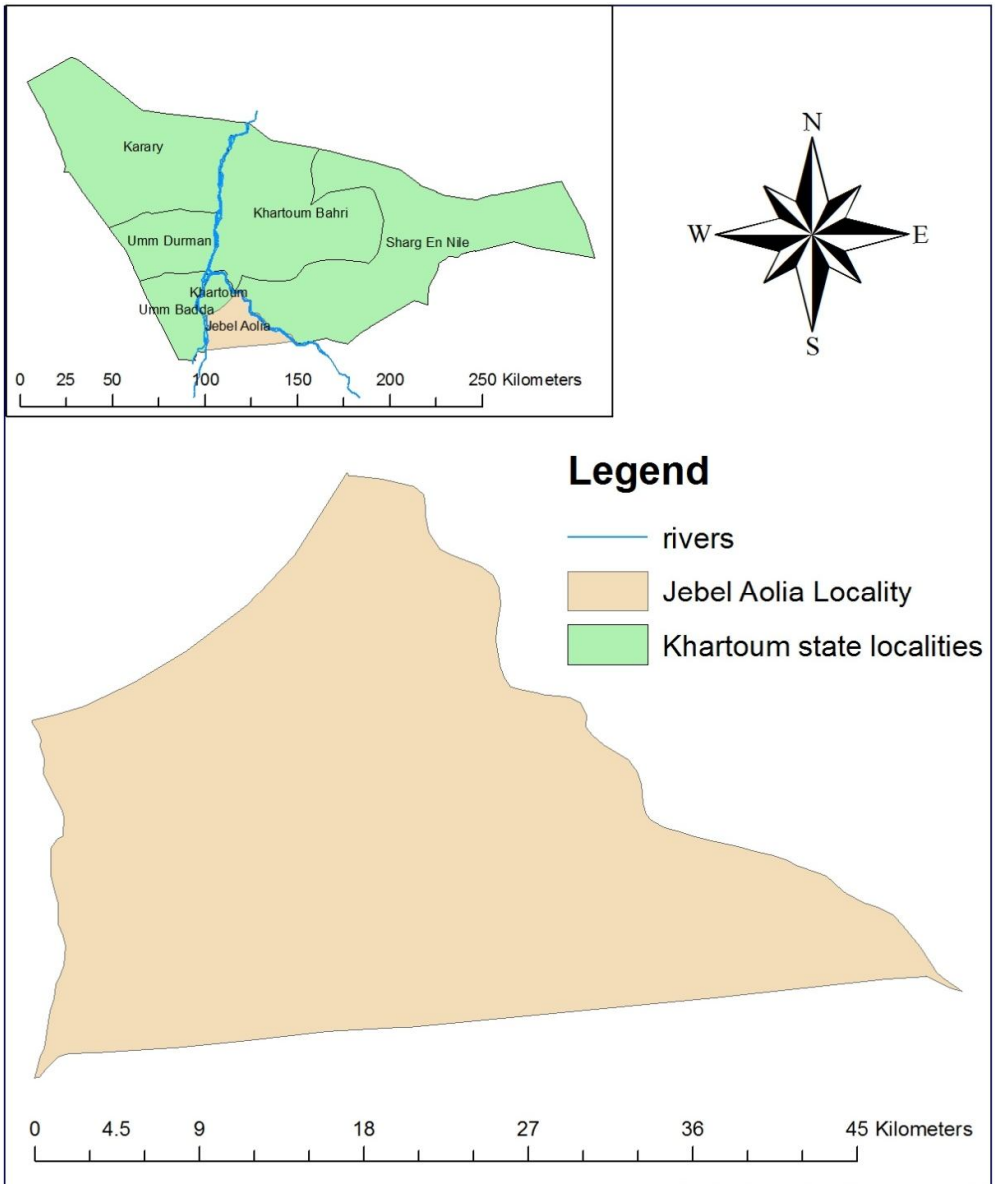

Figure 1:Map of Jebel Aulia locality .

Source: Produced by dr. Alaa aldin Bushra Elsheikh (2014) .

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).

$C = P(1-P)D/SE^2n$

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 were20; 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:

a- Milk yield.

b- Price of milk.

c- Price of replacement heifers.

2.2.2.The secondary sources of data

Ministry of Agriculture and Animal Resources. Federal Ministry of Animal Resource. Different publications .

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 prepare 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 at4 °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 12were left for controls.

3. 20µ1 of the negative control was added to the wells, A12, Bl1, B12, C11 and C12.

4. 20 µl of the positive control were added to the wells F11, F12, G1l, 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 shaked 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 m1 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 rumminants 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).
8. Average annual milk yield (Medani,1996).
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 rest50% 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 breading = 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 was20% (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 and13% (1/8) for sheep (Table 2).

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

Diagnostic tests / Animal species	RBPT			C-ELISA		
	Number of herds examined	+ve herds	%	Number of herds examined	+ve herds	%
Cattle	29	26	90%	29	22	76%
Camels	5	1	20%	5	1	20%
Goats	11	4	36%	11	2	18%
Sheep	8	3	38%	8	1	13%
Total	53	34	64%	53	26	49%

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 and5% (4/84) for sheep. The (C-ELISA) test revealed11% (43/393) overall individual animal prevalence, 19%

((39/207) for cattle, 5% (1/20) for camels, 4% (3/82) for goats and1% (1/84) for sheep (Table 3).

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

Diagnostic test / Animal species	RBPT			C-ELISA		
	Number of samples examined	+ve samples	Prevalence rate%	Number of samples examined	+ve samples	Prevalence rate%
Cattle	207	72	35%	207	39	19%
Camels	20	1	5%	20	1	5%
Goats	82	7	9%	82	3	4%
Sheep	84	4	5%	84	1	1%
Total	393	84	21%	393	43	11%

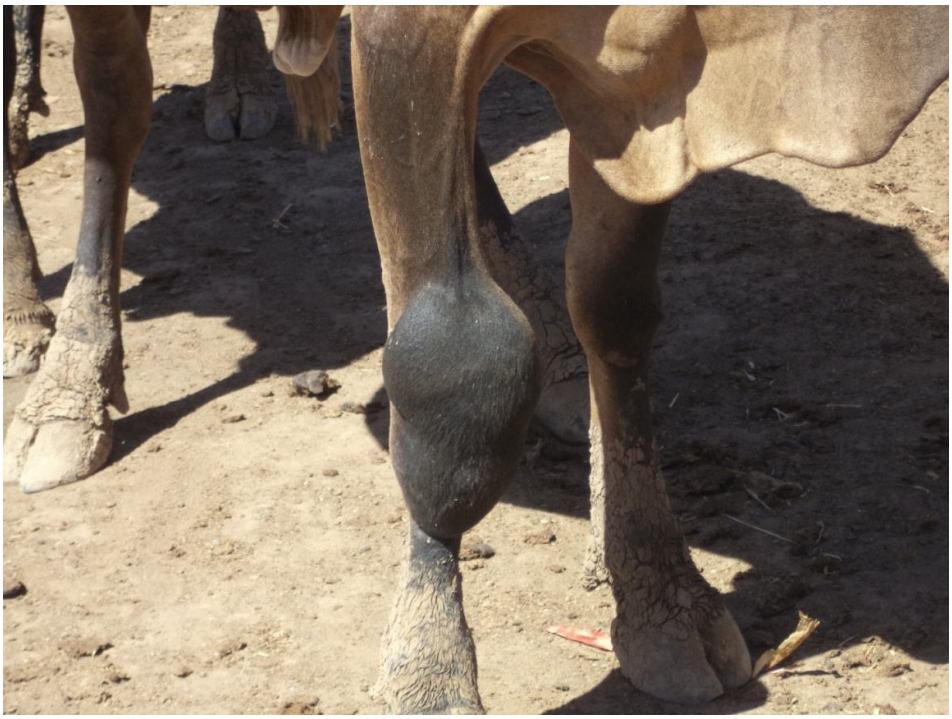

Figure 2 : Hygroma in fore limb

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 that77% 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: Amulti 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 ≤ 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

Risk factor	No. Positive	χ^2	df	P-value
Unit				
Jebel Aulia	17	4.178	1	0.041
Elazhry	9			
Owner education				
Illiterate	15	4.247	2	0.120
Rather good	4			
Well educated	7			
type of the herd				
One species	16	5.44	1	0.020
Multi-species	10			
Dealing with sick animal				
Have veterinarian	23	1.791	1	0.181
No veterinary care	3			
Abortion cases				
No abortion cases	12	5.638	1	0.018
Have abortion cases	14			
Knowing the cause of abortion				
Well knowledge	4	4.197	1	0.041
Bad knowledge	22			
Breeding				
Have a bull	25	1.865	1	0.172
Borrow bull	1			
Feeding and watering animals				
Separate	21	7.483	1	0.006
Together with other species	5			
Source of water				
Have source	25	2.840	1	0.092
Common canal	1			

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 ≤ 0.25in 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).

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

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 x 2,614 L x 20% + 22x 2,614 L x 10%

7,319,2+ 5,750,8 = 13,070 L

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

=13,070 x 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 rest50% 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 foetix price of female calf at weaning + Number of aborted male foeti x price of male at weaning…… (2)

7x 1000 + 7x800 =12,600SDG

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 breading = Number of repeat breeding cows x Cost of repeat breeding due to brucellosis/cow…….. (3) = 3x1, 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 SDG

Total loss in the sample studied =55,530 SDG

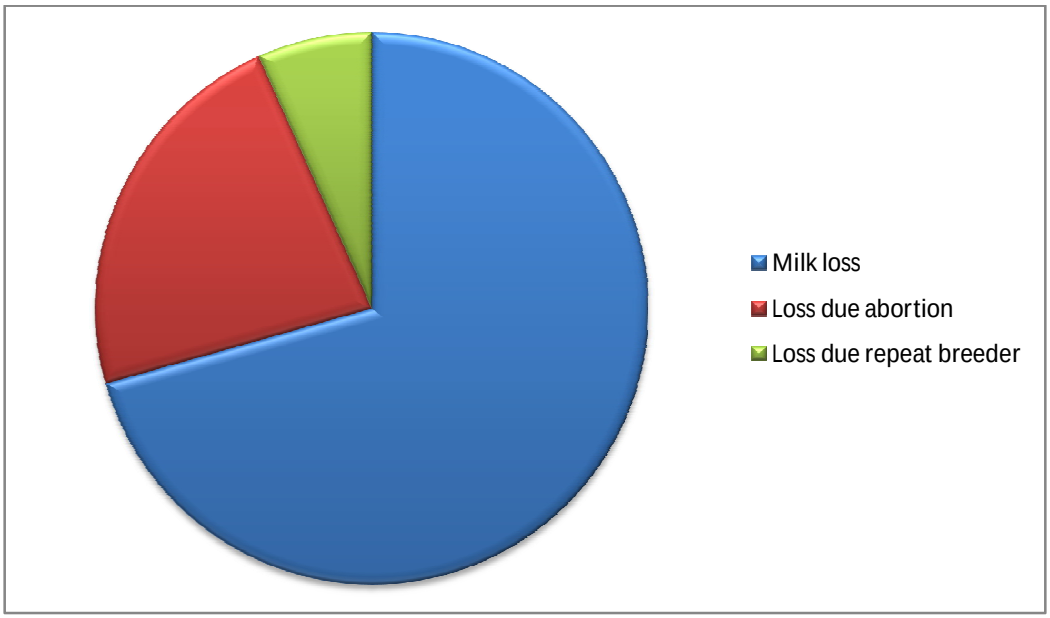

Figure 4: Economic loss due to brucellosis in Jebel Aulia locality

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 x739= 198.245 SDG

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

Total loss in whole locality = 55,53/207x 12,684 =3,402,620 SDG

*2.3 (SDG) per US dollar in (2010) Angara and ELfadil (2014).

*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

Item	SDG	US$
Loss in milk	39,210	8,524
Loss in calve	12,600	2,739

Loss in fertility	3,120	678
Total loss in the sample	55,530	12,072
Total loss in the herds sample	198,245	34,097
Total in the locality	3,402,620	739,700

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 got8.3% 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. Alower 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 was5.6% in goats.

The seroprevalence of ovine brucellosis determined in this study was 5% while the herd prevalence was38%. 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 Eritea. 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 (Alkdru) 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% respectivley 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 wide spread nature of Brucella infection 9.7 % in small rumminants in Afar, Somalic 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 week, 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 investiging 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 Elfadil (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

Abbas, B., Qarawi, A.A. and Al-Hawas, A.(2000). Survey of camel husbandry in Qassim region, Saudi Arabia, *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux,* **53:** 285–292.

Abbas, B. and Agab, H.(2002). A review of camel brucellosis. *Preventive Veterinary Medicine,* **55:** 47-56.

Abdalla, A. (1966). Incidence of animal brucellosis in Wadi Halfa district. Sudan. *Journal of Veterinary Science Animal Husbandry,* **7**: 28-31.

Afzal, M. and Sakkir, M. (1994). Survey of antibodies against various infectious disease agents in racing camels in Abu Dhabi, United Arab Emirates. *Revue Scientifique et Technique de l`office International des Epizooties,* **13:** 787–792.

Agab, H. and Abbas, B.(1999). Epidemiological studies on camel diseases in eastern Sudan, *World Animal Reviews,***92:** 42–51.

Akbarmehr, J. and Ghiyamirad ,M.(2011). Serological survey of Brucellosis in live stock animals in Sarab city (East Azarbagian province), Iran .*A J of Mic.Res* 5(**10):**1220-1223.

Al-Ani, F. K.; Al-Sharrifi M. and Khalil, F. (1998). Serological survey on camel brucellosis in Iraq. Camel Newslett., **14:** 32–33.

Ali,A.A.A.(2013). Epidemiological study on sheep Brucellosis in North Kordofan state, Sudan, MPVM thesis Sudan University of Science and Technology.

Al-Majali, A.M., Talafha, A.Q., Ababneh, M.M., Ababneh, M.M., (2009) Seroprevalence and risk factors for bovine brucellosis in Jordan. *Journal of Veterinary Science,* **10:** 61-65.

Alton, G. G.; Jones, L. M.; and Pietz, D. E. (1975). Laboratory techniques in brucellosis. World Health Organization, Geneva.

Alton, G.G. (1985). Rev. 1 and H38 *Brucella melitensis* vaccines. *Brucella melitensis.* CEC seminar, Brussels, November 1984, 215-227.

Alton, G.G.; Jones, L.M.; Angus, R.D. & Verger, J.M. (1988). Techniques for the Brucellosis Laboratory. INRA, Paris, France.

Alton, G.G., (1990).*Brucella melitensis*: "Animal brucellosis". (Nielsen, K.,Duncan, J. R., eds).*CRC .press*. Boston,: 383-409.

Angara, T. E. (2005). Socioeconomic Aspects of Brucellosis in Kuku Dairy Scheme, Khartoum State, Sudan.*Ph.D. Thesis*. Sudan University of Science and Technology pp 99-102.

Angara,T. E. E.; Ismail ,A. A.; Agab, H. and Saeed N. S.(2009). Sero-prevalence of bovine brucellosis in Kuku Dairy Scheme. Sudan *Journal of Veterinary Science Animal Husbandry*, **48** (1&2) :27-35.

Angara. T.E.; Elfadil. M.H. M.(2014) Economic Impact of Infertility in Crossbred Dairy Cows: The Case of Eastern Nile Locality, Sudan. *Indian Journal of Research* **(3)**:195-197.

Anon (2009). census of the Ministry of Agriculture, Animal Resources and Irrigation, Khartoum State, Sudan.

Anon (2011). Ministry of Agriculture, Animal Resources and Irrigation, Khartoum State, Sudan.

Anon (2012). Reports of the Information Centre, Ministry of Animal Resources, Fisheries and Land Ranges. Sudan.

Anoon (2014).www.cbos.gov.sd.

Anonymous (1986). Joint FAO WHO expert committee on brucellosis: sixth report. Geneva, NY: World Health Organization: 132.

AOAD: Arab organization for agriculture development report**(1995)**. December, 1995, Khartoum, Sudan: 414 -474.

Ariza J.; Pellicer T. ; Pallares R. and Gudiol F. (1992)Specific antibodies profile in human brucellosis. *Clinical Infectious Diseases*, **14**:131-140.

Ashenafi,F.;Teshale,S.;Ejeta,G.;Fikru,R.andLaikemariam,Y.(2007). Distribution of brucellosis among small ruminants in the pastoral region of Afar, eastern Ethiopia. *Revue Scientifique et Technique de l`office International des Epizooties,* 26(**3**):731-739.

Aulakh H. K., Patil P. K. , Sharma S., Kumar H. , Mahajan V.and Sandhu K.S. (2008). A Study on the Epidemiology of Bovine Brucellosis in Punjab (India) Using Milk-ELISA. *Acta Veterinaria Brunensis,* **77**:393-399.

Barroso, G. P., Rodriguez, C. P. R. ; Extremera, B. G. ; Maldonado, M. A.; Huertas, G. G. and Salguero, M. A. (2002). Study of 1,595 brucellosis cases in the Almeria province (1972-1998) based on epidemiological data from disease reporting. *Revista Clínica Española*, **202:** 577-582.

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).

Bennett, S.G.J. (1943). Annual Report of the Sudan Veterinary Service. 29-30.

Bennett,S.; Woods,T.; Liyanage,W.M. and Smith,D.l., (1991).A simplified general method for cluster- sample survey of health in developing countries. *World Health Statistics Quarterly,* **44:** 98-106.

Berhe G., Belihu K. and Asfaw Y. (2007).Seroepidemiological Investigation of Bovine Brucellosis in the Extensive Cattle Production System of Tigray Region of Ethiopia. *The Journal of Applied Research in Veterinary Medicine, **5,** (2)65-71.*

Bertu,W. J. ;Ajogi,I.; Bale J.O.O.; Kwaga ;J.K.P. and Ocholi ,R.A.(2010). Epidemiological of Brucellosis in small ruminants in Platean State Nigeria *A J of Mic Res* 4(**19)**1935-1938.

Blasco, J.M. (1997). A review of the use of B. melitensis Rev 1 vaccine in adult sheep and goats. *Preventive Veterinary Medicine* .;**31**(3-4):275-83.

Bricker, B. J.(2002). PCR as adiagnostic tool for brucellosis. *Veterinary Microbiology*, ***90*:** 435-446.

Cadmus, S.I. B. ; Ijagbone, I . F. ; Oputa ,H. E. ;Adesokan , H . K. and Stack , J. A.(2006). Serological survey of Brucellosis in live stock animals and worker in Ibadan, Nigeria . *Journal of Biomedical Materials*, **9**:163-168.

Capasso, L. (2002). Bacteria in two-millennia-old cheese, and related epizoonoses in Roman populations. *Journal of Infection*,**45:** 122–127**.**

CDC. Centers of Diseases Control and Prevention**(2005).** Brucellosis (*Brucella melitensis, abortus, suis,* and *canis*). Available at: http://www.cdc.gov/ ncidod /dbmd /disease info/brucellosis_t.htm. Accessed 4 Jun 2007.

Chilonda P. & Huylenbrock, G.V. (2001).A conceptual framework for the economic analysis of factors influencing decision-making of small-scale farmers in animal health management, *ev.sci.tech.Off.int.Epiz.*,20 (**3**),:687-700.

Chukwu, C.C. (1987). Brucellosis in Africa: part 1: the prevalence .*Bulletin of Animal* Health and *Production* in *Africa*, **33:** 193-198.

Corbel, M.J. (1973). The direct fluorescent antibody test for detection of *Brucella abortus* in bovine abortion material. *Journal of Hygiene Camb*. **71**: 123-129.

Corbel, M.J. and Hendary. L.F.D. **(1983).** Methods for the identification of *Brucella*. Ministry of Agriculture, Fisheries and Food, London.

Corbel, M.J., Brinley-Morgan, W.J., (1984). Genus *Brucella* Meyer and Shaw 1920,173AL, In Krieg, N.R., Holt, J.G.: Bergey's Manual of

Systematic Bacteriology,Vol.1, Williams & Wilkins, Baltimore-London, 377-388.

Corbel, M.J.and Macmillan, A.P. (1998). Brucellosis. *Bacterial Infections* **3:** 819-820.

Corbel, M .J. (2006). Brucellosis in humans and animals. WHO Press, Geneva, Switzerland .p:89.

Crawford R.P.;Heck F.C.;and WilliamsJ.D.(1978). Experience with *Brucella abortus* strain 19 vaccine in adult Texas cattle *The Journal of the American Medical Association*, 173;1445-1461.

Crawford R.P.; Huber J.D.; Adams B.S.(1990). Epidemiology and surveillance. In: Nielsen K, Duncan JR (eds.). Animal Brucellosis. p. 131-151, CRC Press, Boca Raton.

Dafalla. E.N. and Khan, A.Q. **(1958).** The occurrence, epidemiology and control of animal brucellosis in the Sudan. *Bulletin of Animal* Health and *Production* in *Africa, 6:* 243-247.

Davis, D.S.; Nielsen, K.; Duncan, J.R. (**1990).** Brucellosis in wildlife, in Animal Brucellosis *FL***,** 321-334.

Dawood A. H.(2008) Brucellosis in Camels (Camelus dromedorius) in the south province of Jordan. *American Journal of Agricultural and Biological Sciences* 3 (**3**): 623-626, 2008.

DelVecchio V.G.; Kapatral V.; Redkar R.J(2002). The genome sequence of the facultative intracellular pathogen *Brucella melitensis. Proceedings of the National Academy of Sciences, USA* **99**: 443-8.

Ebrahim ,W. M.O.,(2013) Seroprevalence and risk factors of bvine Brucellosis) in Khartoum State ,Sudan MPVM thesis, Sudan University of Science and Technology.

EC European Commission Regulation**(2002).** No 535/2002. Official Journal of the European Communities **L 80:** 22-28.

El – Ansary E.H., Mohammed A., Hamad A.R.A., Karom A.G.O. (2001). Brucellosis among animals and human contacts in eastern Sudan. *Saudi Medical Journal*, **22**: 557 – 579.

El-Boshy M. ; Abbas H.; El-Khodery S.and Osman S.(2009). Cytokine response and clinicopathological findings in *Brucella* infected camels (*Camelus dromedarius*) *Veterinarni Medicina, 54, (***1***): 25–32.*

El Sanousi, S.M.; Omer, E.E., (1985). Serological survey of brucellosis in Benghazi cow project (Libya). *International Journal of Zoonoses* ,**12**: 207–210.

El-Taweel, A.H.,(1999). Country reports. Brucellosis Information Workshop, Ramallah, 19–20 October.

Esuruoso, G.O.(1977). Control of Brucellosis in Nigeria. Paper presented at the 2nd International symposium on Veterinary Epidemiology and Economic canberva, Australia.

Ewalt D.R.; Payeur J.B.; Rhyan J.C.(1997).*Brucella suis* biovar 1 in naturally infected cattle: a bacteriological, serological, and histological study. *Journal* of *Veterinary Diagnostic* Investigation ,**10:** 417-420.

FAO Food and Agriculture Orgnization **(2003).** "Progress in understanding brucellosis," Vet Record. 641.

Fekete, A.;Bantle, A. J.;Halling, S. M.& Saborn M .R. (1990). Preliminary development of diagnostic test for Brucella using polymerase chain reaction. *Journal of Applied Bacteriology, **69***: 216-227.

Fensterbank, R. (1976). Treatment of cows brucellosis with long acting oxytetracycline. *Annales de Recherches Veterinaires,***7:** 231-240.

FeredeY.;Mengesha,D.;Mekenen,G. and H/melekat,M.(2011)A study and the Seroprevalence of small ruminant Brucellosis in and around Bahir Dar,North West Ethiopia, *Ethiopia Veterinary Journal,*15**(2):**35-44.

Gallien, P.; Dorn, C.; Alban, G.; Staak, C.and Protz, P. (1998). Detection of *Brucella* species in organs of naturally infected cattle by poly merase chain reaction .*Veterinary Record* **142**:512-514.

Gameel, S.E.; Mohamed, S.O.; Mustafa, A.A.; Azwai, S.M.,(1993). Prevalence of camel brucellosis in Libya. *Tropical Animal Health* and *Production,***25:** 91–93.

Geering, W. A.; Forman, J. A. and Nunn, M. J. (1995). Exotic Diseases of Animals. Aust. Gov. Publishing Service, Canberra, Australia, p: 301-306.

Godfroid J. and Kasbohrer A.(2002). Brocellosis in the European Union and the Narway at the turn of the twenty –first century . *Veterinary Microbiology*,**90** : 135-145.

Godfroid J; Bosman P. P.;Herr S.; Bishop G. C. **(2004 a)** Bovine brucellosis. In*: COETZER J A W,TUSTIN R C (eds) infectious disease of livestock, 2nd Edition Vol 3 Oxford University Press* :1510-1527).

Godfroid J.; Garin- bastuj,I . B. ; BLASCO J. N.; Thomson J. ;Thoen C. O. (2004 b). *Brucella melitensis* infection in: Coetzer J A W, Tustin R C (eds) infectious disease of livestock, 2nd Edition Vol 3 Oxford University Press : 1535-1541.

Grilló, M.J.; Barberán, M.; Blasco, J.M. (1997). Transmission of *Brucella Melitensis* from sheep to lambs. *Vet . Rec.***140**: 602-605.

Herr , S. (1994) . *Brucella melitensis* infection In J A W Thomson G R, Tustin R C (eds) Infectious Disease of Livestock with special Refrence to Southern Africa Vol .2. Oxford University Press .Cape Town : 1073-1075.

Higgins, A., (1986). The camel in health and disease, (BailliereTindall, London).

Hoffmann, D. (1999): Asian Livestock to the Year 2000 and beyond. *Working Paper series* **1/2,** 1-44.

Hosie, B.D.; Al-Bakri, O.M.; Futter, R.J. (1985). Survey of brucellosis in goats and sheep in the Yemen Arab Republic: comparison of tests for Brucella melitensis infection in sheep. *Tropical Animal Health* and *Production*, **17**:93–9

Ishag A.M.S.(2013). Epidemiological study of brucellosis in sheep in Khartoum state ,Sudan MPVM thesis, Sudan University of Science and Technology.

Islam A. M., Samad A. M. and. Rahman A. K. M. A (2010). Risk factors associated with prevalence of brucellosis in blackbengal goats in Bangladesh *Bangladesh Journal* of *Veterinary Medicine*,8 (**2**): 141 – 147.

Kaplan , M., (1966). The problems of choice between control and eradication . Joint WHO/FAO Expert Committee Zoonoses , Geneva , Dec.6-12.

King, N. B. (1951). Capillary tube mthod for conducting the Brucella stained antigen milk test. *American Journal of Veterinary Research*, **12:** 75-80.

Lapaque N; Moriyon I; Moreno E; Gorvel JP.(2005). Brucella lipo polysaccharide acts as a virulence factor. *Current Opinion* in *Microbiology*,**8** : 60-6.

Leal Klevezas , D. S; Martínez-Vázquez , O; López-Merino , A ; Martínez-Soriano J P (1995). Single-step PCR for detection of Brucella spp. from blood and milk of infected animals. *Journal of Clinical Microbiology* ;33 (**12)**:3087-90.

Lopes,L.B.; Nicolino, R. and J.P.A. (**2010).** Brucellosis - Risk Factors and Prevalence: A Review. *The Open Veterinary Science Journal*; **4**: 72-84.

Magzub, H.M. (2001). Caprine brucellosis in Khartoum State. *A thesis for the degree of M. V. Sc*. University of Khartoum , Faculty of Veterinary Medicine, department of Preventive Medicine.

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

Mantur, B. G.;Mangolgi, S.; Mulimani, B**.(1996).** *Brucella melitensis* a sexually transmissible agent . *lancet*; **347**: 1763.

Matope, G., Bhebhe, E., Muma, J.B., Oloya, J., Madekurozwa, R.L., Lund, A., Skjerve, E., (2011). Seroprevalence of brucellosis and its risk factors in cattle from smallholder dairy farms in Zimbabwe. *Tropical Animal Health and Production*, **43**:975-979.

McDermott J. J. and Arimi S. M.(2002). "Brucellosis in sub-Saharan Africa: Epidemiology, Control and Impact," *Veterinary Microbiology*, **90** (1–4) :111–134.

Mcmahan, **V. K. (1944).** Brucellosis of cattle. Circular–222. Kansas Agricultural Experiment Station, Kansas State College of Agricultural and Applied Science. Manhattan, Kansas, USA.

Medani, A.M., **(1996).** *Animal resources and Animal Production in Sudan*, U of K : pp: 56.

Megersa B., Biffa D., Abunna F., Regassa A. & Godfroid J. and Skjerve E.(2011) Seroprevalence of brucellosis and its contribution to abortionin cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Tropical *Animal Health and Production*,**43**:651–656.

Metcalf, H.E. **(1986).** Control of bovine brucellosis in the U.S., in Practices in Veterinary Public Health and Preventive Medicine in the U.S. In: Woods, G. T. (ed.), Iowa State University Press.

Mikolon A.B; Gardner, I.A, And Hietala S.K. **(1998).** Risk factors for brucellosis seropositivity of goat herds in the Mexicali Valley of Baja California, Mexico. *Preventive Veterinary Medicine*, **37** (1/4):185-195.

Mohammed M.A; Shigidy M.T; Al juboori A.Y. (2013). Sero-Prevalence and Epidemiology of Brucellosis in Camels,Sheep and Goats in Abu Dhabi Emirate. *International Journal of Animal and Veterinary Advances* 5(**2)**: 82-86 **.**

Moreno, E., Moriyon, I.(2001). *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. In: Dworkin M, Falkow

S, Rosenberg E, Schleifer KH, Stackebrandt I, eds. New York, NY:Springer.

Morgan, W.J.B. (1982). *Brucella abortus,* Brucella abortus, Handbook of bacterial infections in animals. Jena**,** 53-213.

Morris ,R.S.(1999). The application of economics in animal health programmes: A practical guide.*Rev.Tech.Off.Int.Epiz.,***18:**305-314.

Muma, J. B.; Samui, K. L. ; Siamudaala, V. M. ; Oloya, J. ; Matope, G. ; Omer, M. K. ; Munyeme, M. ; Mubita, C. and Skjerve, **E. (2006).** Prevalence of antibodies to Brucella spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock–wildlife interface areas of Zambia. *Tropical Animal Health and Production* **38**: 195–206.

Muma, J.B., Samui, K.L., Oloya, J., Munyeme, M., Skjerve, E.,(2007). Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, **80**: 306-317.

Musa M.T. (2005). Brucellosis in sheep under traditional farming system in Darfur State, Sudan. *Journal of Veterinary Research p*. 20.

Musa, M.T.; ElSanousi, E.M.; Angra ,T.E.E.; Ali, A.A. (2008). Brucellosis, a challenge to veterinarians in Africa: The situation of the disease in the Sudan. The proceeding of the first scientific conference 17 21/8/2008. Khartoum, Sudan pp. 45-56.

Nasri, M. E.(1962). A serological survey for the detection of Q fever antibodies in the sera of animals in the sudan. *Bull Epizoot Dis Afr journal articles.,* **10:** 55-57.

Nicoletti, P. **(1980).** The epidemiology of brucellosis in animals. *Advances in Veterinary Science and Comparative Medicine,**24***: 69-98.

Nicoletti, P. L.(1982). Diagnosis and vaccination for the control of Bruceollosis in the near East. F.A.O. Animal Health and Production. Rome, Italy .

Nicoletti, P., Milward, F.W. (1985). Efficacy of long-acting oxytetracycline alone or combined with streptomycin in the treatment of bovine brucellosis. *The Journal of the American Medical Association,* **187:** 493-495.

Nielson, K. and Duncan, J.R. **(1990).** Animal Brucellosis. BocaRaton.Florida, USA. Press, Vet .Bull. 454.

Nielsen, K., Cherwonogrodzky, J., Duncan, J. R., Bundle, D. R., Nicoletti, P.,Forbes, L. B.(1991). A competitive enzyme immunoassay for diagnosis of bovine brucellosis. United Nations Press, p: 131-l43. ed: Frank, Julius F. Tokyo.

OIE. World Organisation for Animal Health **(2000)** "Bovine brucellosis," in *Manual of Standards for DiagnosticTests and Vaccines*, p: 328–345, OIE, Paris, France, 4thEdition.

OIE, World Organisation for Animal Health **(2004).** Bovine brucellosis. *In Manual of Standards for Diagnostic Tests and Vaccines*. OIE, Paris,p: 409-439.

OIE. World Organisation for Animal Health **(2009).** Bovine brucellosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Office International des Epizooties, Paris.

Olsen, S.C. and Stoffregen, W.S.(2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. Expert Rev Vaccines. **4(6):**915-28.

Omer F.A.; El Sheikh, O.H.; Mohamed Z.A.; Osman H.M.; Suliman H.B.andOsman A.Y. (1989–1990). Survey of Brucellosis among cattle, camels, goats and sheep in the Sudan. *Sudan Journal of Veterinary Research*, **9**:36-40.

Omer M.K.; Skjerve E.; Holstad G.; woldehiwot z.;Macmillan A.P.(2000) . Prevalence of Antibodies to *Brucella* spp in Cattle, Sheep, Goats, Horses and Camels in the State of Eritrea ; Influence of Husbandry Systems. *Epidemiology and Infection***,125**,: 447 – 453.

Omer M.M; Abdelaziz A.A.; Abusalab S.M.A.and Ahmed A.M. (2007). Survey of brucellosis among sheep, goat, camels, and cattle in Kassala Eastern Sudan. *Journal of Animal and Veterinary Advances,*6(**5):**635-637.

Omran E.O.M. (2011). Prevalence of Brucellosis in different animal species and man in Sinnar state, Sudan. *M.V.Sc. Thesis.* Sudan Academy of Science.

Otte,M.J. and Gumm, I.D. (1997). Intra- cluster correlation coefficients of 20 infections calculated from the results of cluster sample surveys. *Preventive Veterinary Medicine,* **31:**147- 150.

Ozekicit, T., Atmaca, S., Akpolat, N., Batun, S. and Elci, S. (2003). Analysis of serum by RBPT and TAT from 20,663 patient in Southeast turkey suspected of having brucellosis. *Brucellosis International Reasearch Conference , University of Navara Pamp lona Spain.*

Pappas, G ; SiozopoulouV ; Saplaoura K ;VasiliouA ;Christou L ;Akritidis Nand Tsianos E .V.(2007). Health litracy in the field of infectious diseases :the paradigm of brucellosis. *Journal of Infection,**54**,40-45.*

Paul ,A. (1980). The Epidemiology of bovine brucellosis. *Advances in Veterinary Science and Comparative Medicine***24: 75.**

Pouillot R.; Garin-bastuji B.; Gerbier, G.; Coche, Y.; Cau C., Dufour B. & Moutou F. (1997). The brucellin skin test as a tool to differentiate false positive serological reactions in bovine brucellosis. *Veterinary Research* ,**28**:365–374.

Put S.N.H.,Shaw A.P.M.,Woods A. J.,Tyler L.and James A., D. (**1988).**Veterinary epidemiology and economics in Africa.Amanual for use in the design and appraisal of livestock health policy .I LCA ,ISBN 92-9053-067-6.

Quinn ,P.J.; Carter, M.E.; Markey, B.; Carter, G.R.(1994). Clinical Veterinary Microbiology. p:261-267, Wolfe Publishing, London.

Radostits, O .M. ;Blood, D . C.; Gay, C.C . (1994) .Brucellosis caused by Brucella abortus (Bangs disease of cattle, sheep, pigs and horses) 8th Edition, the Bath Press, Avon :787-803.

Radostits, O.M.; Gay, C.C.; Blood, D.C.; Hinchcliff, K.W. **(2000).** Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed. W. B. Saunders, London, 1877 p.

Radostits ,O .M.;Gay , C.C.;Hinchcliff, K. W. and Constable,P. D.(2006) .Veterinary medicine .10th edition .Saunders Elsevier ,Oxford .Ch:18pp:963-985.

Radostits, O.M., Gay, C. Blood D.C. and Hinchclift, K.W.(2007). Disease associated with helminthes parasite. In: veterinary medicine, a text book of the(disease of cattle, sheep, goat, pigs and horses). 10th ed. London: Bailliere, Tindall, UK, p: 984-988.

Radwan, A.I.; Hafez, S.M.; Al Aska, A.K.; Al Xamani, M.J.; Bekairi, S.I.; Julaifi, M.A. and Al Mukayel, A.A. **(1987).** Experimental treatment of ovine brucellosis with oxytetracycline alone or combined with streptomycin. XXIII World Veterinary Congress, Montreal Quebec, **14 :**16-21 .

Radwan, A.I.; Bekairi, S.I.; Mukayel, A.A.; Al-Bokmy, A.M.; Prasad, P.V.; Azar, F.N.; Coloyan, E.R., (1995).Control of *Brucella melitensis* infection in a large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev.1 vaccine. *Rev. Sci. Tech*. **14:** 719–732.

Raga, I.O. **(2000).** Studies on Brucellosis in Camel and Cattle in Darfur States. *M.V.Sc. thesis*, Faculty of Vet. Sc. Dept of Microbiology, University of Khartoum.

RahmanS. M., FarukO. M.., Her M. , KimY. J. , Kang I.S. , JungC. S.(2011) Prevalence of brucellosis in ruminants in Bangladesh Veterinary Medicine,***56***, *(**8**): 379–385.*

Refai, M. (1989). Brucellosis in animals in Egypt and its control. Publ., WHO., Mediterranean Zoonoses Control Centre. **25:** 1–3.

Refai M.(2002) Incidence and control of brucellosis in the Near East region. *Veterinary Microbiology,* **90**,: 81-110.

Rijpens, N. P. ; Jannes, G. ; van Asbroeck, M. ; Rossau, R. and Herman, L.M. F.**(1996**) .Direct detection of *Brucella* spp. in raw milk by PCR and reverse hybridization with 16S-23S rRNA spacer probes," *Applied and Environmental Microbiology,* **62(5**) : 1683–1688.

Saad E. G.(2013). Epidemiological study of Brucellosis in camels (*Camelus dromedarius*) in Khartoum State ,Sudan *MPVM thesis,* Sudan University of Science and Technology.

Saergerman, C.; Vo t.-K.O.; De Waele, L.; Gilson D.; Bastin, A.; Dubray, G.; Flanagan, P.; Limet, J.N.; Letesson, J.J. & Godfroid, J. (1999). Diagnosis of bovine brucellosis by skin test: conditions for the test and evaluation of its performance. *Veterinary Research,* **145**: 214–218.

Salman, M.D.and Meyer, M,E.(1984). Epidemiology of bovine brucellosis in the Mexicali Valley, Mexico: literature review of disease-associated factors. *American journal* of *veterinary research* **,45** :1557-1560.

Scacchia, M.D.i.; Provvid., o. A.; Ippoliti, C.; Kefle, U.; Sebhatu, T.T.; D'Angelo, A. & De Massis, F.,(2013) 'Prevalence of brucellosis in dairy cattle from the main dairy farming regions of Eritrea', *Onderstepoort Journal of Veterinary Research* **80(1),** 4 pages. http://dx.doi. org/10.4102/ojvr.v80i1.448.

Schnurrenberger P.R.; Walker J.F.; Martin, R.L.(1975). *Brucella* infections in Illinois Veterinarians. *Journal of the American Veterinary Medical Association* **167**:1084-1089.

Shepherd, A. A.; Simpson B. H. and Davidon R. M.(1979). An economic evaluation of the New Zealand BovineBrucellosis Eradication scheme. Second. *International Symposia on Veterinary Epidemiology and Economics,* P: 443-447.

Shuaibi, A.(1999). Palestinian brucellosis control programme. Country reports. Brucellosis InformationWorkshop, Ramallah, 19–20 October.

Smith C.M., SHerman M.D.(1996) . Brucellosis. *In* : Goat Medicine, Baltimore, Lea and Febiger, :423 – 424

Sohn, A.H.; Probert, W.S.; Glaser, C.A.(2003). Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp.. *Emerging Infectious Disease journal,* **9**:485–488.

Stableforth A.W.and Galloway I.A. (1959). Infectious diseases of animals: diseases due to bacteria. London, UK: Butterworths Scientific Publications, **1**:109-119.

Tesfaye G. , Tsegaye W., Chanie M. and Abinet F. (2011) Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms. *Tropical Animal Health and Production,* **43:**1001–1005

Tesfaye A.; Asfaw Y.; Zewde G. and Negussie H. (2012) . Assessment of Risk Factors and Seroprevalence of Small Ruminant Brucellosisin Adamitulu-Jido-Kombolcha District, Oromia Regional State, *Ethiopia Libyan Agriculture Research Center Journal International* 3 **(2):** 47-52.

Teshale S. , Muhie Y., Dagne A. and Kidanemariam A.(2006) Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia : the impact of husbandry practice. *Revue de Médecine Vétérinaire,* **157(11)** :557-563.

Verger J.M.(1985). *B. melitensis* infection in cattle. *Brucella melitensi.* In:Plommet & Vergers, Eds. Martinus Nijhoff Publ.: Dordrecht-Boston-Lancaster.

Verger, J.M., Grimont, F., Grimont, P.A.D., Grayon, M., (1985) *Brucella,* amonospecific genus as shown by deoxyribonucleic acid hybridization. *International journal of* systematic *bacteriology,* **35**: 292-295.

Warsame I. ; Alemu S.; Temesgen W. and Molla W.(2012) Seroprevalence and Associated Risk Factors of Camel (*Camelus dromedaries*) Brucellosis in and Around Dire Dawa, Ethiopia *Global Veterinaria 8 (5): 480-483.*

W. H. O. (1997). Fact sheet N173 . Geneva, Swizerland in www.who.int/inf-fs/en/fact 173.hrml.

Wray, C. (1975). Survival and spread of pathogenic bacteria of veterinary importance within the environment. *Veterinary Bulletin,* **45**: Abstract 546.

Yesuf,M. ;Alemu,S. ;Temesgen,W.;Mazengic,H. and Negussie,H.(2010). Seroprevalence of ovine Brucellosis in South Wollo,North Eastern Ethiopia, *American- Eurasian. Journal* of Agricultural & *Environmental* Sciences.,**9(3):**288-291.

Young E.J.(1995). An overview of human brucellosis. *Clinical Infectious Disease,***2:** 283-90.

Zinsstag, J., , Roth ,F., Orkhon, D. , Chimed-Ochir G., Nansalmaa , M., Kolar J., Vounatsou, P.,(2005).A model of animal–human brucellosis transmission in Mongolia .*Preventive Veterinary Medicine* pp: 1-19 **.**

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:

Age	Less than one year		1-3 y ears		More than 3 years		Total	
Sex	M	F	M	F	F	M	M	F
Cross								
Local								
Pure Foreign								
Total								

7. Farm Labour

occupation	Number of Name of worker	Name of worker(s)	Wage per month

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 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) N o

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.

l. What is the price of LB of milk?

ll. Price of male kid at weaning

Ill. Price of female kid at weaning.

IV. Average milk production per day.

Apppendix 2

C**ase 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 53herds.

Risk factor	distribution	percent
Unit		
Jebel Aulia	41	77.4
Elazhry	12	22.6
Total	53	100.0
Owner education		
Illiterate	23	43.4
Rather good	11	20.8
Well educated	19	35.8
Total	53	100.0
Source of animal		
Bred in the farm	21	39.6
purchase	32	60.4
total	53	100.0
Type of the herd		
One species	24	45.3
Multi-species	29	54.7
Total	53	100.0
Breed		
Local breed	19	35.8
Cross breed	34	64.2
Total	53	100.0
Deal with sick animal		
Have veterinarian	43	81.1
No veterinary care	10	18.9
Total	53	100.0
Vaccination		
Vaccinated herd	33	62.3
Non vaccinated herd	20	37.7
Total	53	100.0
Type of vaccine		
Including *Brucella*	2	3.8
Not including *Brucella*	51	96.2
Total	53	100.0
Abortion case		
No abortion cases	33	62.3
Have abortion cases	20	37.7
Total	53	100.0
Occurrence of abortion		
Repeatedly	7	13.2

Some times	13	24.5
Total	20	37.7
Knowing the cause of abortion		
Well knowledge		
Bad knowledge	15	28.3
Total	38	71.7
	53	100.0
Special barn for calving		
Have calving place	38	71.7
Havenot calving place	15	28.3
Total	53	100.0
Breeding		
Have abull	48	90.6
Borrow bull	5	9.4
Total	53	100.0
Feeding and watering animal		
Separate	33	62.3
Together	20	37.7
with other species	53	100.0
Total		
Source of green fodder		
Buy it	29	54.7
Grazing land	24	45.3
Total	53	100.0
Source of water		
Have source	47	88.7
Common canal	6	11.3
Total	53	100.0
Prevalence		
Negative	27	50.9
Positive	26	49.1
Total	53	100.0

Appendix 4

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

Risk factor	No. Positive	χ^2	df	P-value
Source of animal				
Bred in the farm	11	.15	1	.695
Purchased	15	4		
Vaccination of *Brucella*				
Vaccinated herd	1	.00	1	.978
Non vaccinated her	25	1		
Special barn for calving				
Have calving place	20	.68	1	.407
Have not calving place	6	7		
Source of green fodder				
Buy it	14	.01	1	.901
Grazing land	12	6		

Appendix 5

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

Risk factor	No. positive herds	Exp(B)	95% C.I for Exp(B)	P-value
Unit Jebel Aulia Elazhry	 17 9	.793	.012-51.372	.913
Owner education Illiterate Rather good Well educated	 15 4 7			.098
type of the herd One species Multi-species	 16 10	.386	.023-6.343	.505
Deal with sick animal Have veterinarian No veterinary care	 23 3	.601	.032- 11.290	.734
Knowing the cause of abortion Well knowledge Bad knowledge	 4 22	.472	.033-6.792	.581
Breeding Have a bull Borrow bull	 25 1	2.463	.059-102.415	.636
Feeding and watering animals Separate Together with otherspecies	 21 5	20.741	.586-734.237	.096