INTRODUCTION

Brucellosis is a one of the highly contagious and most important zoonotic disease in tropical area and asignificant cause of losses in animals (OIE,2009). Animal brucellosis poses barrier to trade in animals and animals products and could seriously impair socio-econamic development, especially for livestock owners (Corbel, *et al.*, 2006).losses due to abortion or stillbirths, irregular breeding, loss of milk production and reduced human productivity are some of the economic consequences of the disease. The reduced human productivity can hardly be measured in medical care. *Brucella* considered as a possible bio-terrorist agent. However, it has never been successfully used in this manner. *Brucella abortus ,Brucella melitensis and Brucella suis* considered as agents of mass destruction and as category B organisms. In animals, bovine brucellosis is characterized by reproductive failure which can include abortion, birth of weak, unthrifty calves, orchitis and\or epididymitis in male. The organism causes abortion in cattle after the fifth month of pregnancy with retention of placenta, metritis and subsequent period of infertility. Brucellosis causes several symptoms in human beings including undulating fever, headaches, weakness and weight loss (Nicoletti 2013). Among *Brucella* species, the most pathogenic for human is Brucella melitensis, followed by *Brucella abortus* and then by *Brucella suis* (Acha and Szfres 2003) The zoonotic nature of the marine *Brucella (Brucella cati*) has been documented in several studies (Brew *et al.*, 1999,Sohn *et al.*, 2003). Brucellosis is suspected when clinical signs are evident (i.e abortion); however, laboratory diagnosis or bacteriological investigation are essential for confirmation of the infection. Several tests are available for serological diagnosis, but not all of them are approved for international trade of cattle are the Rose Bengal Test (RBT) (OIE, 2009).Some species of *Brucella (B.abortus*

,B.melitensis and B.suis) have several biovars, which can be differentiated though culure, serology and polymerase chain reaction (PCR) (Godfroid *et al.*, 2013). Brucellosis is widespread in the world (Benkirane 2006). But its prevalence varies considerably depanding on the area and farming systems concerned (Matope *et al.,* 2009). Brucellosis is commonly transmitted to susceptible animals by direct or indirect contacts (Esuruoso, 1974) .Aborted fetus, placental membrance, vaginal discharge or other fluid present after an infected animals has aborted or calved may be contaminated with *Brucella* organism. Another source of infection is through an infected bull during normal service or artificial insemination, excretion through the colostrum and uterine discharge. Also wild animals infected with brucellosis can transmit the disease to domestic livestock population (Davis *et al.,* 1990; Schelling *et al.,*2003). Therefore the objactives of this study is to assess the epidemiology of the disease in cattle in El fasher.

1-To estimate the prevalence of infection of brucellosis in cattle.

2-To investigate risk factors associated with the disease.

Chapter one

Literature review

1.1. An overview

Brucellosis remains a major zoonosis worldwide. Although many countries have eradicated *Br. abortus* from cattle. In some areas *Br. melitensis* has emerged as a cuase of infection in this species as well sheep and goats. Despite vaccination campaigns with the Rev1 strain ,*Br. melitensis* remains the principal cause of humans brucellosis *Br. suis* is also emerging as an agent of infection in cattle.

1-2. The genus Brucella

It is generally accepted that, the genus *Brucella* consist of small, none motile none sporing ,gram negative cocci,coccobacilli or short rods. 0.5-0.7 µm in diameter and 0.6-1.5 µm in length. It occurs, singly, in pairs (less frequently), short chains or small groups. It is aerobic (carboxyphilic), possessing respiratory type of metabolism and has cytochrome based electron acceptor. Many strains require supplementary CO^2 for growth especially on primary isolation. *Brucella* does not grow under strict anaerobic conditions.It is catalase positive, usually oxidase positive but negative strains occur, reduce nitrate, produce H^2S and hydrolyze urea. Production of indole, acetyl methyl carbinol and methyl red test and utilization of citrate are negative.They do not lyse erythrocytes and do liquefy gelatin or inspissated serum.Colonies on primary isolation on serum dextrose agar (SDA) or other clear media are usually 0.5-1.0 mm in diameter, transparent, raised, and convex with a circular outline and an entire edge.The colonies have shinny surfaces and appear clear pale yellow (honey like in colour), (Hirsh and Zee, 1999).

1.2.1. Taxonomy of brucella and biovars

Classification:

Domain*: *Bacteria

Phylum:* *Proteobacteria

Class:* *Alphaproteobacteria

Order:* *Rhizobiales

Family:* *Brucellosis

Genus:* *Brucella

Species:

B. abortus

B. canis

B. ceti

B. inopinata

B. melitensis

B. microti

B. neotomae

B.ovis

B. pinnipediali, (Meyer and Shaw, 1920).

The history of the genus *Brucella* began with the recognition by Evans (1918) of the similarity of the agent of malta fever reported by Bruce (1887) and later described as *Micrococcus Melitensis* to *Bacterium abortus* the agent of contagious abortion of cattle described by Bang (1897) and the *abortus* like bacteria isolated from swine abortion by Traum (1914) . Three biovars are recongniced for *B. melitensis* (1-3), seven for *B. abortus* (1-7), and five for *B. suis* (1-5).

Species identification is routinely based on lysis by phages and on some simple l To chemical tests such as catalase, oxidase urease, nitrate and H2S production.For *B. melitensis, B. abortus* and *B. suis,* the identification at the biovars leval is currently performed by five main tests, dye (thionin and basic fuchsin) sensitivity, and agglutination with monospecific A and M anti sera and lysis with brucella-specific phages (Alton *et al.,* 1988). Arecently developed co-agglutination test, using latex beads coated with apair of monoclonal antibodies directed against the rough lipopolysaccharide (R-LPS) and 25 *KDa* outer membrance protin (*OMP25),*respectively (Boden *et al.,* 1997) makes it possible to accurately differentiate *B. ovis* from *B. canis* and the occasional rough isolates of the smooth *Brucella* species.*B. melitensis* biovars 3 appears to be the most frequently biovars3, especially its differentiation from biovar 2 appears sometimes equivocal. Due to use insufficiently discriminating monospecific area a number of strains identified initially as biovar 2 were later confirmed as biovar 3 by expert laboratories. *Brucella* strains were islated from carcasses of seals, dolphins, porpoises and whales shown to be associated with abortions and meningocephalitis in several sea mammal species and with pathological impacts in striped dolphins (*Stenella coeruleoalba)* .

1.2.2: Susceptibility to phages

Brucella are highly infectious and facultative intracellular bacterial pathogens causing brucellosis**.** Phages which infect and lyse *Brucella* strains are known for over half a century (Parnas *et al,*. 1958; Brinley-Morgan *et el.*, 1960; Jablonski, 1962). After some basic characterization, a typing set comprising five reference phages [Tb (Tbilisi), Fi (Firenze), Wb (Weybridge), Bk (Berkeley), R/C] was developed (Corbel, 1984). Some years later the typing set was complemented by phages Iz (Izatnagar; Joint FAO/WHO Expert Committee on Brucellosis, 1984) and since then has been used in many diagnostic laboratories worldwide.The same holds true for a set of *Brucella* reference strains serving as control for lysotyping.The original typing set has also been modified by adding other phages, e.g., S708,Bk2,F1,F25, and Np,some which are mutants of the reference phages (Moreira-Jacob, 1968; corbel *et al.*, 1988; Rigby *et al.*, 1989; Hammerl *et al.*, 2014). All Brucella phages described so far have apodoviral morphology and are closely related, demonstrated by restriction analysis and southern hybridization (Segondy *et al.*, 1988; Rigby *et al.*, 1989).They are considered as a single taxonomic species (Corbel and Thomas, 1976; Ackermann *et al.*, 1981).

Over 40 *Brucella* phages have been reported to be lytic for *Brucella* members.All phages are specific for the genus *Brucella*, and are not known to be active against any other bacteria that have been tested. Thus lysis by *Brucella* phages is a useful test to confirm the identity of *Brucella* spp and for speciation within the genus.

1.3: Brucellosis

1.3.1: Definition

Brucellosis is a contagious disease of animals and it is transmissible to man (Radostits *et al.*, 2007, Angara and Shuaib , 2015).

1.3.2: Transmission of the disease

Brucellosis is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their product. It affects people of all age groups and of both sexes. Although there has been great progress in controlling the disease in many countries,there still remain progions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs. It is an important human disease in many parts of the world especially in the Mediterranean countries in Europe north and east Africa, the middle East, south and central Asia and central and south America and yet it is often unrecognized and frequently goes unreported.

Acoording to Buxton and Fraser (1977) the disease is transmitted from infected animals or contaminated materials to susceptible ones through mucous membrance of the alimentary and respiratory tracts, conjunctiva, abraded and intact skin,artificial insemination and through the vagina in some species. Insect could also act as vehicles of infection (Corbel, 1989). In man, is by inhalation,ingestion through conjunctiva and skin.

Infection from a contaminated environment occurs more frequently than is recognized. Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market place, especially if abortions occur .Inhalation of bacteria may then result from contamination of skin or conjunctivae from soiled surfaces. Water

Sources such as wells may contaminated areas.

1.3.3: Pathogenicity

Brucella species are facultative intracellular pathogens that can survive and multiply within phagocytic cells of the host.The mechanisms by which *Brucella* evades intracellular killing are incompletely understood. Nevertheless ,*Brucella* organisms ultimately become sequestered within monocytes and macrophages of the reticuloenthelial system (RES), such as lymph nodes, liver,spleen and bone marrw. Brucellosis is a systemic infection that can involve any organ or tissue of the body. When clinical symptoms related to a specific organ predominate,the disease is termed "*Localized".* Commonly, localization involves organs of the RES.The virulence of *Brucella* varies considerably according to species, strain and the size of infection inoculums. Host susceptibility is also variable and is associated with the reproductive status. Thus,in the field, all intermediate stages between typical acute infection and complete resistance may be observed.In addition,vaccinal immunity may modify the bacteria host relationship.The symptoms,which have been described in cattle are abortion, hygroma, orchitis, retention of placenta, weak or still births and long calving intervals (Blood *et al.*, 1989 and Musa *et al.*, 1990),while in other animals the symptoms are variable.

1.3.4: The disease in animals

Brucellosis is a sub -acute or chronic disease which may affect many species of animals.In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male (OIE, 2006).

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and numbers of organisms

shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder and the lymph nodes which drain the relevant areas. Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs. However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding transmits infection in swine and dogs and, to a lesser exten, sheep and goats. Presistent bacteraemias are also more common in the first two species but is usually intermittent and of short duration (World Health Organization, 2006).

1.3.5: The disease in cattle

It is a world –wide occurrence. Cattle are the most important source of infection with *B. abortus* but other bovideae can be of local importance (Corbel, 1989). The disease is characterized by abortion (most frequently), hygroma, orchitis, placentitis and infertility (Blood *et al.,* 1989). The disease in cattle is widely distriduted, and has been recorded 120 out of the 175 (68.8%) countries of the world (Nielsen and Dunkan, 1990). It has been reported in 101 countries (WHO report, 1992). In Europe, bovine brucellosis has not been reported in some countries (Corbel, 1989). In USA the disease was eradicated from most areas and reduced in some. In Asia, Japan is free from the disease but it has been reported in India. Bovine brucellosis was eradicated from Australia. In Africa, Bovine brucellosis has been reported in most African countries. In Arab countries, the disease has been reported from Syria,Saudi Arabia,Ira,Yemen and all the Arab countries in Africa except Morocco (Thimm, 1982). Both *B. abortus* and *B. melitensis* ware isolated from cattle in many countries. The organisms were isolated from virous sources including milk, hygroma fluids, vaginal swabs, semen (Chatterjee *et al.,* 1995 and Casolinuovo *et al.,* 1996), lymph nodes and aborted fetuses (Musa,*el al.,* 1990).

1.3.6: Epidemiology of Brucellosis in animals

This will vary with the host species affected . For cattle, infection is usually caused by *B. abortus*. However, *B. melitensis* and rarely *B. suis* can also establish themselves in cattle and the mode of transmission is then similar to that for *B. abortus* .These infections are particularly dangerous to humans because of the high virulence of most *B. melitensis* and *B. suis* strains and of the numbers of bacteria that are excreted by these animals.

In cattle and other Bovidae , *Brucella* is usually transmitted from animal to animal by contact following an abortion . Pasture or animal barn may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation , conjunctival , inoculation , skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection.

In sheep and goats , *B .melitensis* is nearly always the infecting species. *B. ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays a greater role. The transmission of disease is facilitated by purchasing animals from unscreened sources (World Health Organization 2006).

1.3.7: Host/ Species Affected

Secondary hosts play a small part if any in the maintenance or spread of a particular *Brucella abortus* mainly infects cattle and is the main cause of contagious abortion in cattle (Manthei and Carter, 1950; Dekeijzer, 1981; Crawford *et al.,* 1990). However sheep, goats, dogs, camels, buffaloes as well as feral animals may also contract *B. abortus.*

Although sheep do not easily become infected with *B.abortus* (Collier and Molello, 1964; Allsup, 1974) they may become carriers and excrete *Brucella* for up to 40 months once they have acquired the infection (Luchsinger and Anderson, 1967).

Isolation of *B. abortus* from swine (Ray, 1979), horses (Robertson *et al.*, 1973), and camels (Al-khalaf and El-khaladi, 1989) in areas with enzootic brucellosis clearly indicates that these species may acquire infection with *B. abortus.* However, their significance as a host for *B. abortus* is doubtful, as these animals species usually do not intermingle with cattle. Dogs with naturally acquired *B. abortus* infection play an important role in the epidemiology of cattle brucellosis (Chary, 1970). The relationship between infected dogs and outbreaks of brucellosis in cattle has not only been reported but has also been demonstrated (Prior, 1976; Forbes, 1990). Although the infections material from the genital tract usually clears after 2-3 months, some infected cattle become carriers of *brucella* and excrete it intermittently for many years (philippon *et al.,* 1970; Herr *et al.,* 1990).

1.3.8: Survival of *Brucella* in the environment

The survival of the organism in the environment may play a role in the epidemiology of the disease. Wray (1975) reviewed many studies conducted to determine the ability of *Brucella* organisms to survive under various experimental and environmental conditions.

Temperature, humidity,and pH influence the organisms ability to survive in the environment.

Brucellae are sensitive to direct sunlight, disinfection and pasteurization. In dry conditions they survive only if embedded in protein (Davies and Casey, 1973).

Brucellae can survive in tap water for several months at 4-8c°, 2.5 years at 0c°, and several years in frozen tissues or medium. *Brucellae* can also survive up to 60 days in damp soil, and up to 144 days at 20c° and 40c° relative humidity.

Brucellae can survive 30 days in urine, 75 days in aborted fetuses and more than 200 days in uterine exudate. In bedding contaminated with infected faecal material *Brucella* will be destroyed at 56-61c° within 4.5 hours (King, 1957). However, there are conflicting reports as to its survival in liquid manure. According to one study *B. abortus* can survive at least 8 months at 12c°(Plommet, 1972). Yet another study indicate that the survival of *Brucella* is subject to seasonal influence. It has been found that *Brucella* can survive in faeces, slurry, or liquid manure 85-103 days in the winter, 120-210 days in spring, 30-180 days in summer and 50-120 days in autumn (Kerimov, 1983). Although *B. abortus* is relatively resistant and may survive for a considerable time, the environment is not considered to be important source of infection(Wray, 1975).

1.4: Diagnosis of Brucellosis

An outbreak of brucellosis is hardly ever confined to one animal and there are no pathognomonic signs. Therefore, clinical examination of aborted material is not of great diagnostic value. Demonstration of characteristic clumps of *Brucella* organisms in stained smears of hygroma, fluid, chorionic epithelium or the use of fluorescent antibody techniques to examine foetal stomach content and uterine, or vaginal exudate may provide a tentative diagnosis (Corbel, 1973)

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk.

1.4.1: Serological tests

Body fluids such, as serum, uterine discharge, vaginal mucus, milk, or semen plasma from suspected cattle may contain different quantities of antibodies of the M, G1 G2, and A types directed against *Brucella* (Beh, 1974). Because infected cattle may or may not produce all antibody types in detectable quantities several tests are used to detect brucellosis.

1.4.1.1: Rose Bengal plate Test (RBPT)

The Rose Bengal plate test is a spot agglutination technique. Because the test does not need special laboratory facilities and is simple and easy to perform it is used to screen sera for *Brucella* antibodies. The test detects specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgM1 than IgM and IgG2 types (Levieux, 1974). The test may yield negative results in infected cattle that give positive results with the CFT (Rose and Roepke, 1957).

Although the low pH (+3.6) of the antigen enhances the specificity of the test, the temprature at which the reaction takes place may influence the sensitivity and specificity of the RB test (MacMillan, 1990).

1.5: Treatment of Brucellosis

Treatment is not usually undertaken. Trials using bovine plasma, sulfadiazine, streptomycin and chlortetracycline given parenterally, and the latter two as udder infection, have been unsuccessful in eliminating the infection. The use of long – acting Oxyteteracycline at 20 mg/kg body weight intramuscularly at 3-4 day intervals for five treatments in combination with Streptomycine at 25 mg/kg body weight intramuscularly or intravenously daily for 7 consecutive days was partially successful in the treatment of infected cow (Ncoletti, *et al.,* 1985). The administration of Oxyteteracycline concurrently with vaccination may reduce the antibody response (Smith, *et al.,* 1983).

1.6: Control and eradication

Bovine brucellosis may be controlled with an effective vaccination program or eradicated using a test and slaughter program. Vaccination using strain 19 will markedly reduce the prevalence of abortion but the level of infection will not be reduced at a corresponding rate. Even with a widespread vaccination program there will be foci of infection which are perpetuated indenfinitely. Complete eradication is the alternative to control by vaccination and some countries have already achieved this status and other are currently engaged in eradication program Hall,(1979). Certain basic consideration apply to all programs aimed at the eradication of brucellosis.

The control programs indigenous to any given area must receive primary recognition, and any plan or plans must be adapted to that area

Cooperation at all levels of government from the local to the national is absolutely essential for the success of a program. Such cooperation is attained only after an intensive program of education has been carried out. The individual owner of an infected herd must come to recognize the problem of brucellosis and express a willingness to cooperate. Experience has revealed that the owner must be impressed with the hazards of the disease for human health and with the economic losses which may be incurred because of infected animals.

A reliable and uniform diagnostic procedure must be generally available. If disease is detected in a herd, established procedures should be available for handling the disease .If immunization is to be carried out, a standardized and effective immunization agent should be readily available. The disposal of infected animals may create a serious economic threat for the owner and the possibilities of indemnity must be explored.

Finally, and of major importance, the movement of animals from one area to another must be controlled at a high level, since a rigid eradication program in one area may be nullified by neglect in a neighboring area (National Research Council, 1977).

1.7: Vaccination

Vaccination with *B.abortus* strain 19 live vaccine is a valuable aid in brucellosis control. Its main value is that it protects uninfected animals living in a contaminated environment, enabling infected animals to be disposed of gradually. This overcomes the main disadvantage of the test and disposal method of eradication in which infected animals must be discarded immediately to avoid spread of infection.Vaccination cannot eradicate brucellosis but can be used to lay the groundwork for eradication. Eradication requires that the infected animal be identified and eliminated from the herd as a source of infection.

Strain 19 *B. abortus* has a low virulence and is incapable of causing abortion except in a proportion of cows vaccinated in late pregnancy, although it can cause undulant fever in man. Its two other weaknesses are its failure to completely prevent infection, especially infection of the udder, and the persistence of vaccinal titers in some animals. The optimum age for vaccination is between 4 to 8 months and there is no significant difference between the immunity conferred at 4 to 8 months of age.

In calves vaccinated between these ages the serum agglutination test returns to negative by the time the animals are of breeding age, except in a small percentage (6%) of cases.

Calves vaccinated with strain 19 at 2 months of age have resistance comparable to those vaccinated at 4-8 month of age (Redman, *et. al.*, 1967). Vaccination of calves with a single dose at 3-5 weeks of age dose not provide protection compared to vaccination at 5 months of age (Plackett, *et. al.*, 1980).

In most control program, vaccination is usually permitted up to 12 months of age, but the proportion of persistent postvaccinal serum and whey reactions increases with increasing age of the vaccinates .

Such persistent reactors may have to be culled in an eradication program unless the reaction can be proved to be the result of vaccination and not due to virulent infection.Vaccination of adult cattle is usually not permitted if an eradication program is contemplated but it may be of value in reducing the effects of an abortion 'storm'. There is no evidence that vaccination of bulls has any value in protecting them against infection. Vaccination has resulted in the development of orchitis and the presenece of *B. abortus* strain 19 in the semen (Lamber, *el al.,*1964*).*For these reasons the vaccination of bulls should be actively discouraged. Strain 19 has been isolated from vaccinated cattle; it is estimated that the organism can be recovered from less than 1:100 000, excluding hypersensitivity cases (Thomas,. *et. al.*, 1981).

Chapter Two

Chapter Two

Material and methods

2.1: Study area

El fasher is a city found in Northern Darfur State, The Sudan. It is located 25E °and 20.96° E latitude 13°N and 37.67°N longitude and it is situated at elevation 748 meters above sea level. Its the capital city of North Darfur region of northwestern Sudan, 195 kilometres (121 mi) northeast of Nyala, Sudan.The town serves as an agricultural marketing point for the cereals and fruits grown in the surrounding region. El fasher is linked by road with both Geneina and Umm-kaddada.In El fasher, the summers are short, sweltering, and partly cloudy; the winter are short, comfortable, windy, and mostly clear; and it is dry year, the temperature typically varies from 52°F to 105°F and is rarely below 45°F or above 105°F. The hot season lasts for 3.0 months, from April to July, with an average daily high temperature above 98°F. The hottest day of the year is May, with an average high of 102°F and low of 85°F.

The cool season lasts for 2.1 months, from December to February , with an average daily high temperature below 88°F. The coldest day of the year is January, with an average low of 52°F and high of 85°F.

The rainfull accumulated over asliding 31-day period centered around each day of the year. El fasher experiences significant seasonal variation in monthly rainfull.

The rainy period of the year lasts for 3.5 months, from June to September , with

asliding 31-day rainfull of at least 0.5 inches. The most rain falls during the 31 days centered around August , with an average total accumulation of 2.9 inches.

The rainless period of the year lasts for 8.5 months, from September 30 to June 13. The least rain falls around December 25, with an average total accumulation of 0.0 inches (https//en.m.wikipedia.org).

2.2: Study design and data collection

The study design was a cross-sectional epidemiological study conducted at the El fasher on North, South and West in El fasher. Randomly selected using multistage sampling strategy was used in the current survey as described by Mortin, *et. al.* (1988) and Thrusfield (2007).

2.3: Sample size determination

The expected prevalence of brucellosis in cattle for the calculation of the sample size was taken from the study in Nigeria (Akinseye, *et al.,* 2016). According to the study on prevalence of bovine brucellosis in cattle the prevalence was estimated about 3.9%

The following formula was used to calculate the sample size Thrusfield (2007). with 95% confidence interval and 5% absolute precision and 3.9 expected prevalence.

Formula: $n = 1.96^2 pex (1\text{-}pex) \div d^2$

Where:

n= sample size

P= expected prevalence

d= desired level of precision

n=3.8416×0.039×0.961÷0.0025=57.59

57.59×4=230 cattle

So number of cattle which will be examined is 230 cattle

2.4: Collection of samples

About 10 ml of blood was withdrawn from the selected animals using a labeled Vacutainer® type tube, put into a wire basket under shade before being taken to laboratory with minimum possible shaking . whole blood sample were taken from selected animals as recommended by OIE, (2007). Five to ten ml of blood were taken from the jugular veins using plain vacutainer tubes. All blood samples were then transported directly to the laboratory of El fasher ,Sudan . Whole blood allowed to clot then the sera were transferred to a labeled Appenorf tube and kept frozen at -20 degree centigrade till used.

2.5: Laboratory procedure

2.5.1: Rose Bengal plate Test (RBPT)

The Rose Bengal pate Test (RBPT) was carried out as described by OIE (2009). The procedure of the test was as follow:1) serum samples and antigen were brought to room temperature first,2) then ,25 ul of each serum sample was place on a plate, 3)an equal volume of antigen was placed near each serum spot,4) serum and antigen were then mixed thoroughly (using a clean wood rod for each sample) to produce a circular or oval zone approximately 2cm in diameter, 5) agglutination was immediately read for after that.

The interpretation of the result was done according to the degree of agglutination, which was recorded as 0, +, ++ and +++. A score of 0 indicated the absence of agglutination ; a score of + indicated barely visible agglutination ; ++ indicated fine agglutination and +++ indicated coarse clumping. Those samples with no agglutination (0) were recorded as negative while other were recorded as positive.

2.5: Data analysis

Results of the study were analyzed using statistical package of social sciences (SPSS). Ch- Square test was used for qualitative data. The significance level was calculated (p,value of 0.005).

Descriptive statistics of the variables were obtained. For each variable (age, sex, breed, body condition, parity and location), frequencies (number of observations within variable) were obtained. Hypotheses of differences of age group, breed sex, and locations between test-positive and test-negative animals were tested by Ch-square test to assess the association between the potential individual and management of risk factor and the outcome variable brucellosis serological status .

Chapter Three

Chapter Three

Results

3.1: Age of animals:

A total of 230 cattle various ages were examined in this study. The presence of the disease in various ages group of cattle.

Table (1) showed the age distribution of cattle , 62 of cattle were calf (age of cattle were less than three year), 70 of cattle were adult (from 4 to 7 years), 98 of cattle were old (mor than 7 years).

Table (1) : Distribution of the age of cattle (n=230) and sero-prevalence of brucellosis in Elfasher City-Northern Darfur.

Age group	**Positive (%)**	**Negative (%)**	**Total (%)**
Calf	5 (2.2%)	57 (24.8%)	62 (27.0%)
Adult	6 (2.6%)	64 (27.8%)	70 (30.4%)
Old	14 (6.1%)	84 (36.5%)	98 (42.6%)
Total	25 (10.9%)	205 (89.1%)	230 (100%)

The result of the study showed that the prevalence of brucellosis in cattle was not significantly (Table 1) lower in calf (less than three years) and adult

(from 4 to 7 years), compared to old cattle (mor than 7 years), It was found that of 205 (89.1%) cattle is no infection . Of 25 (10.9%) cattle were infected.

The Ch-square test showed is not significant association between infection and age of animals. Which is (p-value 0.356).

3.2 : Sex of cattle :

The results of the study showed distribution of 230 cattle examined for brucellosis in El fasher town by sex. A total number of females examined was 173 animals and number of males was 57 animals (Table 2).

Among females 21 animals were found infected (9.1%). Among males, 4 animals were found infected 57 (1.7%) .

Table (2): Distribution cattle sex (n=230) examined for brucellosis in Elfasher City-Northern Darfur.

Infection	Male (%)	Female (%)	Total (%)
Positive	4 (1.7%)	21 (9.1%)	25 (10.9%)
Negative	53 (23.0%)	152 (66.1%)	205 (89.1%)
Total	57 (24.8%)	173 (75.2%)	230 (100%)

Analyzed by the Ch-square test (Table 2) , the results showed not significant association between brucellosis and sex of animals , (p-value 0.281).

However, a higher rate of infection was observed in female animals.

3.3: Breed of animals

The results of the study showed distribution of 230 cattle examined for brucellosis in El fasher town by breed. Total number of cross breed examined was 66 animals and number of local breed was 164 animals (Table 3).

Among cross breed 7 animals were found infected (3.0%). Among local breed, 18 animals were found infected (7.8%)

Table (3): Distribution of cattle breed (n=230) examined for brucellosis in Elfasher City- Northern Darfur.

Infection	**Cross (%)**	**Local (%)**	**Total (%)**
Positive	7 (3.0%)	18 (7.8%)	25 (10.9%)
Negative	59 (25.7%)	146 (64.5%)	205 (89.1%)
Total	66 (28.7%)	164 (71.3%)	230 (100%)

Ch-square test showed there was no significant association between brucellosis and breed of animals (p-value 0.935).

3.4: Body condition:

Table 4 showed the distribution of cattle brucellosis in El fasher town, in 230 cattle examined according to body condition . Good body condition cattle found 9 was infected animals (3.9%), and poor body condition found 16 was infected (7.0%) .

Table (4): Distribution of cattle body condition (n=230) examined for brucellosis in Elfasher City-Northern Darfur.

Infection	Good body condition (%)	Poor body condition(%)	Total (%)
Positive	9 (3.9%)	16 (7.0%)	25 (10.9%)
Negative	133 (57.8%)	72 (31.3%)	205 (89.1%)
Total	142 (61.7%)	88 (38.3%)	230 (100%)

The prevalence (Table 4), of cattle brucellosis significantly lower in good body condition compared to poor body condition, It was found that of 142

cattle good body condition ,9 were infected (3.9%). And 88 poor body condition, 16 were infected (7.0%).

The Ch-square test showed significant association between infection and body condition of cattle which is significant (p-value 0.005).

3.5: Area (Localities) :

The result showed that the over all prevalence (Table 5), out of 230 there was 25 (10.9%) of cattle have brucellosis. The highest rate of infection was in south of city (7.4%), but the west (3.0%), and the north (0.4%) where lowerst.

Table (5): Sero- prevalenc of Cattle brucellosis in El fasher directions- Northern Darfur.

Infection	**South (%)**	**North (%)**	**West (%)**	**Total (%)**
Positive	17 (7.4%)	1 (0.4%)	7 (3.0%)	25 (10.9%)
Negative	53 (23.0%)	44 (19.1%)	108 (47.0%)	205 (89.1%)
Total	70 (30.4%)	45 (19.6%)	115 (50.0%)	230 (100%)

The result of the study showed that there was highly significant association between animals and various area, (p-value 0.000) .

Table (6): Univariate analysis for risk factors of brucellosis in cattle (n=230), using the Ch-square test in El fasher City-Northern Darfur.

Risk factor	No Tested	No Positive (%)	Df	X^2	p-value
Age					
Calf	62	5 (2.2%)	2	2.066	0.356
Adult	70	6 (2.6%)			
Old	98	14 (6.1)			
Sex					
Male	57	4 (1.7%)	1	1.161	0.281
Female	173	21 (9.1%)			
Breed					
Cross	66	7 (3.0%)	1	0.007	0.935
Local	164	18 (7.8%)			
Body condition					
Good	142	9 (3.9%)	1	7.867	0.005
Poor	88	16 (7.0%)			
Origin					
South	70	17 (7.4%)	2	19.194	0.000
North	45	1 (0.4%)			
West	115	7 (3.0%)			

Chapter Four

Discussion

Chapter Four

Discussion :

Brucellosis is one of the disease that have drawn attention and concern at it causes economic losses in cattle, besides to its zoonotic dimension Radostits *el al.,* (2007). The disease can be diagnosed using rose Bengal plate test.

In this study, the results showed that the prevalence rate of cattle brucellosis in cattle in El fasher area was 10.9% based on RBPT. The high prevalence rate was in south in El fasher area and this could be attributed to the fact that it has a very large numbers of livestock ,but the low prevalence rates was in north and this might be attributed to fewer number of animal compared to other areas.The prevalence rate was similar to those reported in the country especially in El dein area and area with similar husbandry method . Musa (990), found that the prevalence rate of the disease in cattle in some close locality, and heist 22.2% in Idd El Firsan locality, 9.6% in Nyala locality,12.8% in Wadi Salih locality, 8.8% in Zalingei locality . Yousif (2010) reported 10.3% prevalence rate of the disease in West Darfur state . Shigidi (2010) reported in different states of the Sudan and prevalence rate of the disease ranges from 3-40%.

The study revealed that, brucellosis appears to be widely spread in El fasher area . The existence of brucellosis in the Al dein coupled with the lack of control measures especially in the traditional sector which maintain the vast majority of animal wealth in Darfur. Methods of husbandry were found to be the major factors responsible for the spread of the disease. The results are lower than another study carried out in Khartoum state. In Guinea Bissau the prevalence was 18.6% , in India the a prevalence of the disease was 18.3%

The sero-prevalence of brucellosis by origin (localities) has investigated in this study. The rate of infection in South was 7.4%, in West was 3.0%, and in north was 0.4%. There is significant association between the brucellosis and localities of the animals (p-value 0.000), so which highly significant.The higher rate of infection was in South (7.4%) fallowed by West (3.0%) , this could be attributed to the fact that it has a very large numbers of livestock.Age of animals is factor investigated in this study. Our study showed that the rate of brucellosis was 2.2% in age of less than 3 years, 2.6% in age from 4 to 7 years and 6.1% in old cattle. Our study showed on significant association between brucellosis and age of animals examined (p-value = 0.356) .Our study showed that female have higher rate of infection than male cattle ,the rate of infection in females was 9.1% while in male animals was 1.7%. However, there was no significant association between bruucellosis and sex of animals (p-value = 0.281)

The results of our study showed that the prevalence of brucellosis in cattle within different body score of the animals was: 3.9% in good body score, 7.0% in poor body score, However, there was significant association between brucellosis and body condition of animals (p-value = 0.005). this could be attributed to the fact that brucellosis is high disease which may affect the general health of the affected animal.

Our study showed that local breed have higher rate of infection than cross breed , the rate of infection in local breed was 7.8% while in cross breed was 3.0% . However, there was no significant association between infection and breed of animals (p-value = 0.935).

The prevalence of brucellosis relation to location (area) was: 7.4% in South , 0.4% in North and 3.0% in West . Higher rate in South compared with North and West. However, there was high significant association between

brucellosis and area of animals, this could be attributed to the fact that it has a very large numbers of livestock.

Conclusions and recommendations:

Conclusions:

The output of this study indicates, that the overall sero-prevalence of brucellosis was 10.9% . The distribution of the prevalence of brucellosis by age showed that the prevalence in old animals is higher than in adult and calf, (6.1% in old animals (> 7 year), adult 2.6% and calf (< 3 year) 2.2%. For body score, the prevalence of cattle brucellosis is high in animals in poor body score (7.0%) and low in animals in good body score (3.9%).

Distribution by sex, the prevalence of brucellosis was 9.1% in females and 1.7% in male. The prevalence of cattle brucellosis according to the geographical areas of cattle was higher in South (7.4%), and low in North (0.4%) and West (3.0%).Distribution of cattle brucellosis by breed of animals was: local breed is high (7.8%) than cross breed (3.0%).

Uusing the Ch- squre, analysis showed a highly significance regarding the localities (area of animals).

Recommendations:

- Numbers of samples used in this study were small compared to the animal population sampled, so, its recommended that, samples sizes should be representative in further researches.
- Regular monitoring programs that lead to disease control strategy which required reducing the economic impact and public health consequences of brucellosis.
- Due to lack of public health awareness and extension programs in this area, work should be directed to human brucellosis to evaluate the impact of the disease on the public health.
- Vaccination programs should be attempted to control the disease.
- There should be co ordinations with the related authorities in the Republic of Chad to determine the magnitude of spread of the disease in the areas around the border to adopt effective control programs in these areas.