Introduction

Brucellosis, also known as "undulant fever, Mediterranean fever" or "Malta fever" in Human is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It affects people of all age groups and both sexes. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals, and consequently, transmission to the human population frequently occurs (*Corbel et al., 2006*).

The disease in the Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia and Central and South America and often unrecognized and frequently goes unreported. There are only a few still occur in people returning from endemic countries (*Corbel et al., 2006*).Brucellosis is defined as a contagious bacterial primarily of ruminants, characterized by inflammation of the genital organs and foetal membrane. Abortion, sterility, and formation of localized lesions in the lymphatic system and joints (*Cadmus, et al., 2006*).

In female animals, the bacteria are localized in the udder followed by excretion via milk and in male animals orchitis and epididymitis can lead to infertility, *(Gwida et al., 2010).*Brucellosis is a contagious infectious disease caused by bacterial species of the genus *Bruclla.* Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis,* and occasionally by *B. suis.* Infection is widespread globally. Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent, *(OIE, 2009).*

Brucellosis is generally introduced into herds by infected animals. The organism localizes in the reproductive organs and/or udder. Infected animals may shed high numbers of bacteria in milk, aborted fetuses, vaginal discharges, placental membranes, and birth fluids. Susceptible animals can become infected via ingestion of pasture, feed, or water contaminated with these excretions. Artificial insemination with infected semen can result in infection of the recipient cow. In swine, natural breeding is an important method of transmitting the disease. Horses generally acquire the infection through contact with infected cattle or swine. Dogs usually become infected by ingesting contaminated fetuses, placentas, or milk. Dog-todog transmission is rare, *(AVMA, 2007).*

Infection in pregnant cows is characterized by abortion, birth of dead or weak calves, retained placenta, endometritis, repeat breeding, infertility as well as reduction or complete loss of milk yield after the abortion. In bulls the disease result in testicular lesions such as orchitis, epididymitis, and seminal vesiculitis which affect their breeding capacity *(Chatikobo et al., 2008).*Brucellosis was first recognized as disease affecting human on the island of Malta in the 19th and early 20th centuries. *Brucella* organisms can be found worldwide, but brucellosis is more common in countries having poorly standardized or ineffective animal and public health programs. Biovarieties vary with respect to geographic region. *B. abortus*, *B.ovis* and *B,canis* are widespread. *B.melitensis* and *B. suis* are irregularly distributed. *B. neotoma* infection of humans or domestic animals has not been reported, and its distribution appears to be limited, *(AVMA, 2007)*.

Brucella abortus mainly affects cattle but occasionally other species of animals such as sheep, swine, dogs, camels and horses may be infected. Animal susceptibility to brucellosis depends on their natural

resistance, age, sex, level of immunity and environmental stress *(Ahmed, 2009)*.The species of *brucella* and their major hosts are *Br. Abortus* (cattle) *Melitenisi* (goats), *Br. Suis* (swine) and *Br. Ovis* (sheep). *Br.abortus* also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen *(Radostits et al., 2000)*. From public health view point, brucellosis is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians *(Gul and Khan, 2007)*.

Most laboratory-acquired brucellosis cases are caused by **.***B. melltensis*, but infection with other strains has also been reported. Most exposures are caused by unsafe laboratory practices. Domestic and wild animal reservoirs may serve as sources of infection of livestock and humans *(AVMA, 2007).*

In the Sudan, the brucellosis occurs in all animal species including wildlife and humans. *B. abort*us *biovars 1,3,6 and 7 and B.melitensis* biovars 2 and 3 were found associated with the disease *(Musa et al., 2008).* There are indications of introduction of some of these biovars through exportation of infected or latently infected animals. Occurrence of *B.melitensis* and *B. abortus* in different animal species throughout the country has important implications in control of the disease *(Musa et al., 2008).*

Brucellosis in cattle was reported in all parts of the Sudan and the prevalence rate was found to be higher in cattle compared to other species Brucellosis is a major veterinary public health challenge, as animals are almost exclusively the source of infection to people. It is often undiagnosed in both human patients and animal sources and it is widely

acknowledged that the epidemiology of Brucellosis in human is poorly understood, particularly in sub-sahara Africa *(Ahmed, 2009)*.

The high prevalence in Africa is due to close human-animal contacts, food consumption customs and the fact that many countries have not yet started control or eradication schemes *(Ahmed, 2009)*,

According to Staak (1990), brucellosis perhaps is the most wide spread and economically important disease in tropical and sub-tropical regions. The direct loss of meat (as a result of abortion, infertility and weight loss) in infected herds of cattle was estimated to be 15% while that of milk (reduced milk production) was 20%.

Brucellosis is an important disease of cattle and an important zoonosis worldwide. It is of major economic importance in developing countries that have not a national brucellosis eradication program. The prevalence of infection varies considerably among herds, areas, and countries. Many countries have made considerable progress with their eradication programs and some have eradicated the disease. However, in other countries brucellosis is still a serious disease problem facing the veterinary and medical professions *(Radostitis et al., 2000).*

Infection occurs in cattle of all ages but is most common in sexually mature animals, particularly dairy cattle. Abortions occur most commonly in outbreaks in unvaccinated heifers after the fifth month of pregnancy. Bulls are affected with orchitis, epididymitis, and seminal vesiculitis *(Radostitis et al., 2000).*

Scientific Justifications:

The impact of brucellosis on public health and livestock reproduction:-

Brucellosis can be of major importance, primarily because of decreased milk production in aborting cows. The common sequel of infertility increases the period between lactations, and in an infected herd the average intercalving period may be prolonged by several months. In addition to the loss of milk production, there is the loss of calves and interference with the breeding program. This is of greatest importance in beef herds, where the calves represent the sole source of income. A high incidence of temporary and permanent infertility results in heavy culling of valuable cows, and some deaths occur as a result of acute metritis following retention of the placenta *(Radostitis et al., 2000).*

In infected cattle populations' brucellosis might lead to a lower calving rate due to temporary infertility and/or abortion, resulting in a decreased milk production cows, increased replacement costs as well as lowered sale value of infected cows. In fully susceptible herds, abortion rates may vary from 30% to 80% *(Karimuribo et al., 2007)*.

General economic losses, however, go far beyond the financial losses suffered by cattle producers alone. These losses include:-

.Losses due to abortion in the affected animal population .1

Diminished milk production, *Brucella* mastitis and contamination .2
,of milk

.Cull and condemnation of infected animals due to breeding failure .3

.Endangering animal export trade of a nation .4

Human brucellosis causing reduced work capacity through sickness .5
.of the affected people

.Government costs on research and eradication schemes .6

.*(*Losses of financial investment, *(Mangen et al., 2002* .7

Objectives:

The objectives of this study were:-

To determine the prevalence of bovine brucellosis in Western .1
.Equatoria State

.To investigate the risk factors associated with bovine brucellosis .2

CHAPTER ONE

LITERATURE REVIEW

Brucellosis:

The historical synonyms of the disease in animals and human-beings:

In 1897, a Danish veterinarian, L.F, Benhard Bang, discovered Bang's bacillus or bacillus of Bang's disease (brucellosis in cattle). Bang's bacillus was not recognized as being related to *Micrococcus melitensis* (isolated by Bruce) until 1918, when Alice Evans in the Hygiene Laboratory of the U.S. Public Health Service (now the National Institutes of Health) showed the close relationship between the two organisms and renamed the genus *Brucella* to honour Bruce *(Sriranganathan et al.,2009).*

In 1914, Traum isolated *B. suis* from an aborted pig foetus in U.S. The description of isolates from cattle and swine led to the recognition of

widespread distribution of the disease. In 1953, a different strain, thought to be a rough *Brucella mutant,* was described in sheep in New Zealand by Buddle and in Australia by Simmons. Although the Subcommittee on the Taxonomy of *Brucella* of the International Committee on Bacteriological Nomenclature was not satisfied that the organism was a member of the genus *Brucella* and advised further study, the species was eventually recognized as *B.ovis,* (*Sriranganathan et al.,2009).*

In 1957, Stoenner and Lackman isolated *B. neotoma* from desert wood rat (*Neotoma lepida)* in Utah, U.S. Carmichael isolated *B. canis* in 1966 from beagles in the U.S. Brucellosis in mammals was first described in 1994 in the U.S. when a bacterial isolate from the aborted foetus of a bottlenose dolphin (*Tursiops truncates)* was characterized as non typical *Brucella spp.* Since 1994, several new *Brucell* species been isolated from marine mammals, (*Sriranganathan, et al., 2009).*

The zoonotic nature of marine *brucella* and its ability to cause abortion in cattle were documented. The discovery of the marine *Brucella* has changed the concept of a land-based distribution of brucellosis and associated control measures to that of land- and ocean-based approach for control and eradication, (*Sriranganathan, et al.,2009).*

As of 2006, eight *Brucella* species are recognized. Six of them infect terrestrial animals: *B. abortus, B. melitensis., B.suis, B. canis and B. neotomae* and two infect marine mammals: *B.cetaceae and B. pinnipediae*. Within these species, seven biovars are recog-nized for *B.abortus,* three for B. melitensis and five for B.suis, the remaining species have not been differentiated into biovare, (*Sriranganathan, et al.,2009).*

Brucella was discovered and isolated for the first time from human in 1887 before it was recognized as an animal pathogen in 1905. The first recognized human case of brucellosis in the USA was in an army officer based in Puerto Rico in 1898. The zoonotic nature of *B. canis* was reported in 1975 in US, (*Sriranganathan, et al.,2009).*

The zoonotic nature of marine brucellae was documented in 1999 in a case of a laboratory-acquired human infection. *B.suis* was the first biological agent to be weaponised by the US in 1942 during its offensive biological warfare program. The agent was formulated to maintain long-term viability, placed into bombs and tested in field trials during 1944-1945 using animal tragents, (*Sriranganathan, et al.,2009).*

By 1967, the USA terminated its offensive program for the development and deployment of *Brucella* and other pathogens as biological weapons *B.melitensis, B.suis and B. abortus* are listed as potential bioweapons by the Centers for Disease Control and Prevention, because of their virulence in humans. This is due to the highly infectious nature of all three species as they can be readily aerosolized (*Sriranganathan, et al.,2009)*.

Moreover an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza.In comparison to abortions, orchitis, followed by persistent infections of supra-mammary lymph nodes and reticuloendothelial system in animals. Human develop symptoms that start out as flu-like symptoms followed by undulant fever with severe cold sweats in between (*Sriranganathan, et al.,2009)*.

In some affected individuals the disease could be fatal if untreated, while others can become permanently infected and suffer from fever and cold

sweats, particularly when they are stressed. Brucellosis has also been associated with mild to sever cases of arthritis in adults and children (*Sriranganathan, et al.,2009).*

Definition of the disease:-

Brucellosis is an infectious, contagious, and worldwide spread of an important zoonosis disease caused by bacteria of the genus *Brucella*. In animals, the disease primarily affects cattle, sheep, goats, swine, and dogs, and is characterized by abortion or infertility and also affects people and other animal species, the disease is characterized by intermittent fever, chills, sweating, headache, myalgia, arthralgia, and a diversity of nonspecific symptoms *(Tun, 2007).*

Morphology and Characteristics of Brucella:

Brucella species are small (0.6 x 0.6 to 1.5 pm), nonmotileconnobaillary, Gram-negative bacteria. As they are not coluorized by 0.5% acetic acid in the modified Ziehl-Neelsen (MZN) staining technique, they are classed as MZN-positive. In MZN stained smeares of body fluids or tissues, they characteristically appear as clusters of red coccobacilli. *(Quinn et al., 2011).*

For taxonomic purposes, all Brucella species should be classified e.g *Brucella meltiensis* as DNA hybridization studies have shown that the genus contains only one species. *Brucella* species are aerobic, capnophsilic and catalase-positive. Apart from *Brucella* species are urease-positive except *B. ovis. Brucella ovis* and some biotypes of *B.abortus* require 5 to 10% Co2 for primary isolation. Moreover, the growth of other Brucella species is enhance-need in an atmosphere of

Co2. Media enriched with blood or serum are required for culturing *B. abortus* biotype 2 and *B.ovis.* Recently, brucellae have been detected in sea-mammals, *(Quinn et al., 2011).*

Brucella grow best on typticase soy-based media or other enriched media with typical doubling time of 2 hours in liquid culture. Although *B. melitensis* bacteremia can be detected within I week by using automated culture systems, 24 culture should be maintained for at least 4 weeks with weekly subculture for diagnostic purposes, *(Purcell et al., 2008).*

Species and biovars are differentiated by their carbondioxied requirements; ability to use glutamic acid, ornithine, lysine, and ribose; production of hydrogen sulfide; growth in the presence of thionine or basic fuchsin dyes; agglutination by anti-sera directed against certain lipopolysaccharide (LPS) epitopes; and susceptibility to lysis by bacteriophage. *Brucella* can grow on blood agar plates and does not require X or V factors for growth, *(Purcell et al., 2008).*

Virulence and pathogenicity:-

The establishment and outcome of infection with *brucellae* depend on the number of infecting organisms and their virulence and also on host susceptiblility *Brucellae*, which lack the major outer-membrane lipopolysaccharide, produce rough colonies and are less virulent than those derived from smooth colonies. Although smooth and rough organisms can enter host cells, rough forms are usually eliminated unlike smooth forms which aim persist and multiply *(Quinn et al., 2008).*

Virulent when engulfed by phagocytes on mucous membranes, are transported to regional lymph nodes. *Brucellae* persist within macrophages but not within neutrophils. Inhibition of phagosome-lysosome function is a major mechanism for intracellular survival and an

important determinant of bacterial virulence. However, many of the mechanisms used by *brucellae* to survive within macrophages are not fully elucidated. Various stress protcins are thought to allow the organisms to adapt to harsh conditions encountered within macrophages *(Quinn et al., 2008).*

From epidemiological evidence, *B. abortus, B. melitensis, and B. suis* have distinct host preferences and the organisms are capable to cause an infection in a wide range of host species, including human. The remaining three members of the species have much greater host specificity. Typically, in all host species *Brucella* grows intracellulary, producing a variable bacteraemic phase followed by localization in the tissues of the genital tract and in the mammary gland. Abortion is typically the first clinical sign of the pregnant female, and orchitis and epididymitis are typical clinical sign of the male *(Tun, 2007).*

In particular, female animals that have reached sexual maturity are most susceptible to infection. It is usually detected in pregnant females through abortions (England, 2004). *Brucellae* can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract. In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the submucosa. Organisms are rapidly ingested by polymorpho-nuclear leukocytes, which generally fail to kill them, and are also phagocytosed by macrophages Bacteria transported in macrophages, which travel to lymph nodes, liver spleen, mammary glands, joints, kidneys, and bone marrow *(Purcell et al., 2008).*

In macrophages, *brucellae* inhibit fusion of phagosomes and lysosomes, and replicate within comparements that contain components of endoplasmic reticulum via a process facilitated by the IV secretion system. If unchecked by macrophage microbicidal mechanisms, the bacteria destroy their host cells and infect additional cells. *Brucellae* can also replicate extracellularly in host tissue, *(Purcell et al., 2008).*

Histopathological, the host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis *(Purcell et al., 2008).*

Description of Brucella abortus species:-

The *brucellae abortus* is the member group of the *brucellae.* In 1897, it was discovered by the Danish veterinarian Bernard Bang who isolated the organism from cows with an infected abortion. Cattle are the natural hosts of the organism but it can also infect other animals. The organisms are gram-negative, coccobaccilli or short rods, in length from 0.8-1.5 pm long and in breadth by 0.6-0.8 pm wide. This species is catalase and oxidase positive and requires carbon dioxide for growth. It produces hydrogen sulphid from sulphur containing amino acids or protein (Stack and MacMillan. 2003), (FAO/OIE).

***Brucella* infection in human beings:-**

The disease in man in the Sudan was first reported in 1904 in Berber in the North of the country (Haseeb, 1950), followed by another incident in Blue Nile (Simpson, 1908), but Erwa (1966) was the first to isolate *B. abortus* from an infected person. From 1928-1937, 311 human cases were reported by medical practitioners in the previous nine provinces of the country then and also 224 cases between 1950-1955 (Dafalla, 1962). Later, *Omer et al (1977)* reported prevalence of (14.7%) in veterinary

workers and their families were serologically positive for the disease. Al Sharif (1994) reported 10% prevalence rate in abattoir workers and milkers in Omdurman abattoir and dairy farms in Khartoum North. Musa (1995) examined 372 pepole in Darfur and found that 49 (13.2%) of them were positive for the disease. Osman (2004) Surveyed eight states in the country from 1998-2002 and reported 99 cases of the disease.

Brucellosis in humans is usually associated with the consumption of unpasteurized milk and soft cheese made from the milk of infected animals, primarily goats, infected with Brucellae melitensis and with occupational exposure of laboratory workers, veterinarians and slaughter hous workers (Tun, 2007). Some vaccines used in livestock, most notably *B. abortus* strain 19. Most of the human brucellosis cases are caused by *B. melitensis* but *B.abortus alsoaccounts for some (Tun, 2007).*

The occurrence of the disease in humans is largely dependent on the animal species, when brucellosis exists in sheep and goats; it causes the greatest incidence of infection in humans (Shresth et al., 2004).

Transmission of brucellosis to humans:-

In humans, brucellosis often occurs through contact with infected animals or materials and through skin abrasions. Human brucellosis was once through to be predominantly transmitted through animal contact. However, it is now being increasingly realized that animal products such as milk and meat products are frequently the source of disease transmission. Dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts, and ice-cream may contain a high concentration of the bacteria and consumption of these is an important cause of brucellosis, (*Kumar, 2010*).

It is the commonest mode of transmission in Khinning stillborn lambs and kids and aborted fetuses, which may be heavily contaminated with *Brucella spp.,* also presents a high risk of brucellosis. Other means of infection include inhalation of airborne animal manure particles. Inhalation is often responsible for a significant number of cases in abattoir employees, (*Kumar, 2010*).

In addition, laboratory-acquired *Brucella* infection due to accidental ingestion, inhalation and muosal or skin contact is a major health hazard for laboratory workers handling cultures of the virulent or attenuated strains. The disease has been recognized as one of the common laboratory-transmitted infections and has been reported to occur in clinical, research, and production laboratories, (*Kumar, 2010*).

Another issue of concern in the use of *Brucellae* as a biological weapon, although there is no reported case of bio-terrorism using *Brucella spp.* Nevertheless, *Brucella* are not difficult to grow and disperse (the American military weaponized *Brucella suis* in 1954). The transmission to humans may result in prolonged illness and long-term sequelae, (*Kumar, 2010*).

Pathogenesis

Brucella spp are facultative intracellular pathogens and establish infection by invading macrophages and evading macrophage induced host protection mechanisms. Following exposure in humans,the organisms travel along the lymphatic pathways; focal disease is most commonly identified in the reticuloendothelial tissues such as the liver and spleen.In chronic infections, organisms typically localize in joints, especially large joints such as the sacroiliac or lumbar vertebral joints. Pulmonary disease is a less common form of brucellosis, (Gul and Khan, 2007).

Clinical Manifestation

After exposure to the bacteria, clinical manifestations may appear within

5 to 60 days. Most infected patients ersent with acute disease consisting of general symptoms, such as fever, malaise, sweats and lymphadenopathy and / or hepatsplenomegaly. However, a subset of patients develops chronic brucellosis, a more severe form of the disease that can be associated with osteo-articular signs including spondylitis, arthritis and osteomyelitis, or genitourinary changes, such as orchtis, epididymitis, glomerulonephritis and kidney abscesses. Life threatening complications comprise in descending order of frequency, neurobrucllosis, liver abscesses and endocarditis. (Xavier et al., 2010)

In humans,brucellosis often occur through contact with infected animals or materials and through skin abrasions.

Diagnosis:

The diagnosis of human brucellosis cannot be made only on clinical grounds due to the wide variety of clinical manifestations of this disease, and it is essential to perform bacteriological and serological tests. However, all physicians dealing with a febrile patient living in an endemic area or recently traveled to a country where brucellosis is endemic must be aware of the possibility that the patient could be infected with brucellosis. For this reason, correct clinical history taking is essential to orientate the diagnosis and the need for some basic questions must be emphasized. Moreover, a rapid screening test must be performed. The Rose Bangal plate test can be used as a sensitive rapid test (Corbel,2006).

Treatment:

Human brucellosis cases are treated with doxcycline and rifampin for a minimum of three to six weeks. Several months may be required for recovery. Relapses occur in approximately 5% of cases. Veterinarians or other animal healthcare workers that are inadvertently inoculated with the Rev-B.melitensis,S19B.abortus, or RB51B.abortus strain vaccines should seek medical attention, and postexposure treatment with doxycycline with or without the addition of rifampin is recommended, (AVMA,2007).

Brucella Infection of Cattle:

Infection is usually caused by B.abortus.However,B.melitensis and rarely B.suis can also establish themselves in cattle (Corbel,2006).

Brucellosis in cattle is usually caused by biovars of B.abortus. In some countries, particularly in southern Europe and western Asia,where cattle are kept in close association with sheep and goat, infection can also be caused by B.melitensis. Occasionally, B.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(Lopes et al.,2010).

Transmission:

Sources of infection for the transmission of bovine brucellosis are aborted fetuses, foetal membranes and vaginal discharges and milk from infected animals. The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, water and licking after birth, fetuses and new born calves, all of which may contain a large number of the organism and constitute a very important source of infection,(Ahmed,2009).

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 organism 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(Corbel,2006).

Pathogenesis:

After ingestion of organism, the bacteria travel through the oral mucosa to the regional lymph nodes. Infection leads to bacteremia,which is usually transient, the organism ultimately settle in the reproductive tissues or musculoskeletal system. Venereally transmitted organism establish chronic infections in the testes and epididymides, Infection of the reproductive tissues of females of these species may occur, (kathleen and Lyn,2008)

Clinical manifestation:

In cattle, *B.abortus* causes abortions and stillbirth, abortion usually occur during the second half of gestation. Some calves are born alive but weak and may die soon after birth. The placenta may be retained and secondary metritis can occur. Lactation may be decreased . After the first abortion, subsequent pregnancies are generally normal, however, cows may shed the organism in milk and utrine discharges. Epididymitis, seminal vesiculities, orchitis or testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or orchitis. Hygromas, particularly on the legs joints, are a common symptom in some tropical countries. Athritis can develop in some long-term infections. Systemic signs do not usually occur in uncomplicated

infections and deaths are rare except in the fetus or new born. Infection in nonpregnant females are usually asymptomatic,(OIE,2009).

Diagnosis:

The clinical picture is not pathognomonic, although the herd history may be helpful, Unequivoal diagnosis of Brucella infections can be made only by the isolation and identification of brucella, but in situation where bacteriological examination is not practicable, diagnosis must be based on serological methods. There is no single test by which a bacterium can be identified as Brucella. A combination of growth characteristics, serological, bacteriological and molecular method is usually needed(FAO,2009).

Prevention and Control:

Treatment of bovine brucellosis is not permitted, all infected cattle and contact which have been exposed to infection, must be slaughtered (Defra et al., 2004). Vaccination of Livestock is crucial to the control of brucellosis. Effective reduction of disease in livestock through mass vaccination will eventually lead to reduction of brucellosis in the human population (Henk et al., 2004).

To reduce exposure , appropriate disposal methods should be instituted for all placentas, birth fluids, fetal membranes and aborted fetuses. Those handling fetal membranes, fluids, placentas and aborted fetuses should use appropriate hygienic practices. Access to potential infected animals should be restricted and newly acquired animals should be quarantined. Consumption of unpasteurized milk products should be avoided, unless

the milk products have been aged for more than 60days and have low moisture content. Masks, gloves and protective eyewear should be worn when handling infected animals (AVMA,2007).

Dairy surveillance programs include bulk tank sampling 2-4 times per year. Herds that are not used for milk production are monitored by blood tests of animals at market or slaughter (AVMA,2007).

Brucella species are susceptible to heat, sunlight and commonly used disinfectant. Brucella organism may survive for up to 6weeks in dust and up to 10weeks in water and soil. Used the stain S19 is an attenuated strain of Brucella, it does retain a mild degree of virulence and is capable of inducing disease(AVMA,2007).

Epidemiology:

Brucellosis is a zoonotic disease, hence the ultimate source of infection are infected animals. The species are the major food-production animals; cattle, sheep, goat,pigs, camels and others, but they can be significant local sources of infection in some regions. The infection has also been identified in marine mammals, including Dolphins, Porpoises and Seals, and may present an emerging hazard to persons occupationally exposed to infected tissues from them (corbel,2006).

The organism is normally associated with infection in sheep and goats, but other species, including dogs, cattle and camels can be infected. In some countries, particularly in the Middle East, *B.melitensis* infection of cattle has emerged as an important problem. Contrary to some traditional views, *B.melitensis* remains fully virulent for man after infecting cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be producted by an individual animals and because of the extensive environmental contamination that

even single abortions or infected birth can produce. B.abortus is the most widespread cause of infection, but associated with much less human disease (Corbel,2006).

B.canis is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild. *Brucella* infection occurs in many species of wild animals but these are rarely implicated as sources of human disease. Brucellosis is an important disease among cattle and remains a major source of disease for humans and domesticated animals. If the countries do not implement high-quality and effective public health and domestic animal health programs as well as a national brucellosis control and eradication program, brucellosis will remain the most common form of an important zoonosis disease in worldwide. Bovine brucellosis caused mainly by B.abortus has a major economic impact on developing countries (Michael, 1997).

FAO,WHO, OIE consider brucellosis the most highly spread zoonosis in the world. The importance of this highly contagious disease is due to; Its economic impact on the animals industry, which effects adversely the supply of animal proteins and the enormous danger to human health through either direct contact with infected animals or the consumption of contaminated milk and dairy products (Tun,2007).

Even though many countries have extensive eradication programs, some even eradicated the disease, brucellosis is still a serious disease challenging the veterinary and medical professions. The reported incidences and the prevalence of the disease vary widely from country to country (Radostits et al, 2000).

Estimated prevalence of antibodies to *Brucella abortus* infection in cattle in Moshi, Tanzania by serum agglutination test in April. Sera were obtained from 417dairy/local cattle of all ages, sexes and breeds were kept then 113 smallholder farms selected randomly. The majority of cattle were kept under zero grazing regimes. The overall prevalence of antibodies to *brucella abortus* were 12.2 and 41,9 for individual cattle and farms, respectively. The rate based on the age seroprevalence profile was estimated at 3.2 per 100 cattle. Using random effect logestic regression model as an analytical method, feeding cotton seed cake, sex, source of animals and levels of exotic blood were found to be associated with seroppostivity to *brucella abortus***(Swai et al., 2003**).

Screened 1106 livestock sera from pastoral and agro-pastoral farming system for *Brucella spp* using Rose Bengal Plate Test. Brucella antibody was detected in all study districts and overall herd seroprevalence of 11.2 were recorded from the study areas. In pastoral area the prevalence of brucellosis was 15.2% where as in agro-pastoral 4.1%. The study revealed that pastoral animals were more than three times more likely of being exposed to brucellla infection compared to animals in the agro-pastoral farming system. Cattle in pastoral farming system had significantly higher brucella antibodies ($P^{\smallsetminus}0.05$) compared to agro-pastoral farming system. Prevalence rate of 12.2% was observed in female animals and 9.8% in male animals. The study showed that *brucella antibodies* was present in both pastoral and agro-pastoral area of East Showa Zone of Oromia Regional State **(Dinka and shala., 2009)**.

Conducted a cross-sectional epidemiological study in cattle. Out of 153 sera tested, 31male and 122 females, 31(1.96) were positive by RBPT. The positive cattle were all females, pregnant and above 5 years old. The overall prevalence of tested animals is 1.96%. The sample were

collected from 6 different areas of cairo , all the cattle owners admitted to drinking raw milk, since they don't know that the disease is zoonotic. They don't also use any protective gear while handling aborted feoteses. There was no vaccination of brucellosis ever done around the province**(Ahmed et al., 2009)**.

A cross-sectional study on different governorates representing all over Egypt to evaluate the potential major risk facto. The prevalence of brucellosis was determined in the ruminants (buffaloes, cattle, sheep and goats) of five different districts viz. Bagerhat, Bogra, Gaibangha, Mymensingh and Sirajgonj of Banglades(**Kaoud et al., 2010)**.

A total of 550 sera samples of 105 buffaloes, 188 cattle, 127 goats and 130 sheep were screened by RBT and were further confirmed with I-ELISA. A structured questionnaire was used to collect epidemiological information on the animals. The overall serological prevalence derived from the samples was 2.87% in buffaloes, 2.66% in cattle, 3.15% in goats, and 2.31% in sheep. The prevalence was relatively higher in females than that in males in cattle, goats and sheep but, an insignificantly higher prevalence was observed in males than that in females in the case of buffalo. A significant association was found between abortion or age and occurrence of brucellosis ($P < 0.01$).

The results of the study provide (a) a comparison of the prevalence of brucellosis in different livestock species in Bangladesh, (b) constitute baseline data for further study of Brucella infections, and (c) are a starting point for the control of brucellosis**(Rahman et al., 2011)**.

Brucellosis is an infectious and zoonotic disease of worldwide distribution. Despite its control program,the disease is endemic in Iran and remains one of the most important public health problems. The aim

of this survey was to determine the seroprovalence of brucellosis in livestock animals in Sarab City,Iran.

A total of 1500 animals (600 cattle, 740 sheep and 160 goats) were examined for brucellosis from February 2007 to September 2008. The examined animals were divided into two sex groups (male andfemale). Moreover cattle were divided into four breed groups (Holstein, Brown Swiss, Native and Mixed). Serological examinations including Rose Bengal plate test (RBPT), serum agglutination test (SAT) and 2-mercaptoethanol test (2ME) were performed on serum samples obtained from examined animals. In overall, out of 1500 blood samples 61 (4.06%) were positive for brucellosis. The prevalence of brucellosis in cattle, sheep and goats were found 3.66, 4.18 and 5%, respectively. The prevalence rates of brucellosis in different breeds of cattle, Holstein, Brown Swiss, native and mixed breeds were determined as: 4.72, 2.22, 2.50 and 3.75%, respectively. The prevalence rates of the disease in male and female animals were determined as follows: Male cattle, 1.53%, female cattle, 3.92%, male sheep, 2.8%, female sheep, 4.89%, male goats, 2.22%, and female goats, 6.08%. There were differences in the prevalences of brucellosis in different breeds and sexes of examined animals however statistically were not significant ($P>0.05$, $2<3.84$). The results of the present study indicated that the prevalence of brucellosis in livestock animals in Sarab City is relatively high and effective control program of the disease should be recommended **(Akbarmehr and Ghiyamirad, 2011**).

A total of 1623 cattle sera were serially tested using the rose Bengal test as screening and complement fixation test as confirmatory tests. The Stata survey command was used to establish prevalences for

the overall and individual variables, while potential risk factors for seropositivity were analyzed using a multivariable logistic

regression analysis. The results showed that 3.5% (95% CI = 2.4, 4.5%) of the animals and 26.1% (95% CI = 18.6, 33.7) of the herds tested had antibodies against *Brucella* species. Village level seroprevalence ranged from 0% to 100%. A higher seroprevalence was observed in pastoral system than mixed farming although this variable was not

significant in the final model. The final logistic regression model identified herd size; with large (odd ratio (OR) = 8.0, 95% CI = 1.9, 33.6) and medium herds (OR = 8.1, 95% CI = 1.9, 34.2) showing higher risk of Brucella infection when compared to small herds. Similarly, the odds of Brucella infection was higher in cattle aged above 4 years when compared to age groups of 1-2 (OR = 5.4, 2.1, 12.9) and 3-4 years (OR = 3.1, 95% CI = 1.0, 9.6). Herd level analysis of the risk factors revealed that large and medium herds as well as herds kept with multiple livestock

species were at higher risk of acquiring *Brucella* infection. Brucellosis in traditional livestock husbandry practices certainly poses a zoonotic risk to the public, in consequence of raw milk consumption, close contact with animals and provision of assistance during parturition. Due to lack of diagnostic facilities and information on its occurrence, human brucellosis is most likely misdiagnosed for other febrile diseases prevailing in the areas and treated empirically**(Megersa et al., 2011b**).

A cross-sectional study was carried out in a village in Menufiya Governorate of Egypt. In June and July 2009, 107 households were selected using systematic sample and all lactating cattle and buffalo present in the household were sampled and tested for antibodies against *Brucella* spp. In addition, a questionnaire collecting information on

potential risk factors for *Brucella* spp. infection in cattle and buffalo was administered to the household member responsible for rearing the livestock. Between December 2009 and February 2010 households were revisited and a second questionnaire regarding KAPs associated with brucellosis was administered.

True individual and household seroprevalence were estimated to be 11.0% (95% CI: 3.06% to 18.4%) and 15.5% (95% CI: 6.61% to 24.7%), respectively. Cattle and buffalo kept in a household with sheep and goats had 6.32 (95% CI: 1.44 to 27.9) times the odds of testing seropositive for *Brucella* spp., compared to cattle and buffalo that were not. Most participants in the study stated that livestock owners assist in the parturition of ruminants without wearing gloves and that some farmers sell animals which they suspect are *Brucella* infected to butchers or at market. Many participants made their livestock's milk into cheese and other dairy products without pasteurising it **(Holt et al., 2011)**.

A cross sectional study was conducted to investigate seroprevalence of brucellosis and the associated risk factors in cattle from smallholder dairy farms in Gokwe, Marirangwe, Mushagashe, Nharira, Rusitu and Wedza areas of Zimbabwe (Matope et al.,2011). A total of 1440 cattle from 203

herds were tested serially for Brucella antibodies using Rose Bengal test (RBT) and the competitive ELISA (c-ELISA). Weighted seroprevalence estimates were calculated and risk factors in individual cattle investigated using logistic regression analysis. The overall individual animal brucellosis seroprevalence was low, with mean of 5.6 % (95 % CI: 4.4 %, 6.8 %). Gokwe had the highest individual (12.6%; 95 % CI: 3.9 %, 21.4 %) and herd-level (40.0%; 95 % CI: 22.1%, 58.0 %), while Wedza had the lowest individual (2.3 %; 95 % CI: 0 %, 5.3 %) and herdlevel (8.0%;

95% CI: 0.0 %, 18.9 %) brucellosis seroprevalence, respectively. In individual cattle, the area of origin, age and history of abortion were independently associated with brucellosis seroprevalence. While the seroprevalence was independent of sex, it decreased with increasing age. Cattle 2-4 years old had higher odds (OR = 3.2; 95 % CI: 1.1, 9.1) of being seropositive compared to those > 7 years. Cows with a history of abortion were more likely to be seropositive (OR= 7.9; 95 % CI: 3.1, 20.1) than controls. In conclusion, the area-to area variation of brucellosis may be linked to ecological factors and differences in management practices. The implementation of stamping out policy, bleeding and testing animals before movement and promoting the use self-contained units are likely to significantly reduce the public health risks associated with Brucella infections in cattle**(Matope et al., 2011)**.

Limited data are available on the risk factors responsible for the occurrence of brucellosis amongst different cattle production systems in Nigeria despite its significant impact on livestock production. Consequently, a cross-sectional study was conducted to determine the prevalence of bovine brucellosis in three cattle production systems in Yewa Division of Ogun State, south-western Nigeria.

A total of 279 blood samples (sedentary = 88; transhumance = 64; trade = 127) were examined for antibodies to *Brucella* sp. using the Rose Bengal test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA). Overall, 24 (8.6%) and 16 (5.7%) of the animals tested seropositive for *Brucella* using RBT and cELISA, respectively. The herd seroprevalences based on RBT and cELISA were 31.6% and 15.8%, respectively. The results using cELISA reveal higher seroprevalence in

the trade cattle (7.9%; confidence intervals [CI] = 3.2% – 12.6%) and those in a sedentary system (5.7%; CI = 0.9% – 10.5%) than in cattle kept under a transhumant management system (1.6%; CI = 1.5% – 4.7%). Age (> 3 years; $p = 0.043$) and breed (Djali; $p = 0.038$) were statistically significant for seropositivity to brucellosis based on cELISA, but sex (female, $p = 0.234$),production system (trade and sedentary; $p = 0.208$) or herd size (> 120; $p = 0.359$) was not. Since breeding stock is mostly sourced from trade and sedentary cattle, it is important that routine serological screening should be conducted before introducing any animal into an existing herd(**Cadmus et al., 2010**).

A study was conducted in the Luwero and Nakasongola districts in central Uganda to determine and compare the prevalence and distribution of antibodies against *Brucella abortus* in cattle under contrasting husbandry practices, using two serological tests. Three hundred and fifteen serum samples were systematically sampled from 29 farms and subsequently tested using the Rose Bengal plate test (RBPT) and Indirect Antibody Enzyme Linked Immunosorbent Assay (I-ELISA). The overall prevalence of antibodies against *Brucella abortus* in the Nakasongola and Luwero districts was 2.4% and 4.7% on RBPT, compared with 1.2% and 3.34 % on I-ELISA. There was no significant difference between the results obtained by RBPT and indirect antibody ELISA ($p > 0.05$). It was noted that antibodies against *Brucella abortus* were widely spread over different farms regardless of the cattle grazing system ($p > 0.05$). Based on the findings, it is feasible to use RBPT as a cheaper screening alternative for brucellosis. A comprehensive national brucellosis study should be undertaken to study the epidemiology and prevalence of brucellosis in Uganda(**Kungu et al., 2010**).

A cross-sectional study in which 791 sheep, 383 goats, 188 cattle milk tanks and 173 buffalo milk tanks were randomly selected in 40 villages and tested for the presence of antibodies against *Brucella* spp. The seroprevalence among different species was estimated and visualized using choropleth maps. A spatial scanning method was used to identify areas with significantly higher proportions of seropositive flocks and milk tanks. We estimated that 12.2% of sheep and 11.3% of goats in the study area were seropositive against *Brucella* spp. and that 12.2% and 12% of cattle and buffalo milk tanks had antibodies against *Brucella* spp. The southern part of the governorate had the highest seroprevalence with significant spatial clustering of seropositive flocks in the proximity of its capital and around the main animal markets(**Hegazy et al., 2011**).

A cross-sectional study, assessed and mapped the seroprevalence of brucellosis in small-scale dairy farming in an urban and peri-urban area of Tajikistan and investigated factors associated with seropositivity. As urban and peri-urban farming is both an opportunity to improve the livelihood for small-scale farmers and a potential public health hazard, studies are warranted to reveal possible peculiarities in the epidemiology of brucellosis in this type of dairy farming. In total, 904 cows of breeding age belonging to 443 herds in 32 villages were serologically tested with indirect enzyme-linked immunosorbent assay (ELISA) and positive samples confirmed with competitive ELISA. Two logistic regression models were used to investigate an association between seropositivity and risk factors at herd and individual level. The herd and individual seroprevalences were 4.1 and 2.0 %, respectively. Herds with a history of abortions were found to be associated with seropositivity [odds ratio (OR) = 5.3; 95 % confidence interval (CI), 1.3–21.3]. Large herds with more than eight cattle were more likely to be seropositive compared to smaller

herds with one to two cattle (OR = 13.9; 95 % CI, 1.6–119). The number of calves produced per cow (indicating age) was found to be associated with seropositivity. Younger cows with one to two produced calves were less likely to be seropositive compared to older cows with more than six produced calves (OR = 0.24; 95 % CI, 0.06–1.0). Neither introduction of new cattle to the herd nor communal grazing was associated with seropositivity. This study shows that infection with *Brucella* (1) is present in small-scale urban and peri-urban dairy farming in Tajikistan and (2) has significant negative effects on reproductive performance in this farming system and (3) that some previously known risk factors for seropositivity in rural farming system were absent here(**AL-Majali et al., 2009)**.

Between April and June 2008, 998 serum samples from 205 herds located in 10 different sectors within the Nyagatare district were screened for brucellosis using Rose Bengal Plate test. Out of a total of 998 serum samples tested, 99 (9.9 %) reacted positive for brucellosis using the Rose Bengal Plate Test (RBPT). Bovine brucellosis was detected in nine out of the ten sectors in Nyagatare, and out of the 205 herds studied, 62 were seropositive. The overall brucellosis herd prevalence rate (HP), i.e. at least one positive RBT reactor identified in aherd, was associated with sector (X2 = 8851.228, P = 0.000), Breed (X2 = 413.567, P= 0.002), and parity of the cow (X2 = 580.292, P = 0.000). Significantly higher brucellosis herd prevalence values were reported for Byera (100 %), Katabagemu (45.45 %), and Rwimbogo (42.86 %) sectors. The herd prevalence was 29.62 % in Ankole cattle (95 % CI: 28.36 to 30.87) and 23.71 (95 % CI: 17.23 to 30.19) in purebred Friesian-Holstein cattle, with a statistically significant difference (x2 = 413.567, P = 0.000). Individual animal prevalence (IAP), i.e. number of individual positive reactors,

differed ($P < 0.05$) between and within the sectors, and was also associated with the breed of the cow. Significant higher overall IAP´s were found in Byera (20 %), Rwimiyaga (12.17%), andRwimbogo (12 . 00 %). Individual animal prevalence was 9.75 % (95 % CI: 9.34 to 10.16) in Ankole cattle and 7.15 % (95% CI: 5.46 to 8.84) in Purebred Friesian-Holstein cattle with a statistically significant difference ($x2 = 335.339$, $P = 0.000$). There was no statistically significant difference in individual prevalence between Ankole cows and crossbred cows. On the other hand, the prevalence of brucellosis in cattle was also found to be higher in the older parities than younger ones. Overall seropositive reactors recorded were 12/204 (5.9 %) for parity 1, 20/181 (11.05 %) for parity 2, and 11/77 (14.29 %) for the fourth parity cows. However, no statistically significant difference was observed in the prevalence of brucellosis between male and female animals. Overall, the study reveals that bovine brucellosis is endemic in Nyagatare. The public health and

livestock productivity implications of the present findings are discussed(**Chatikobo et al., 2008**).

The prevalence of brucellosis was investigated in cattle, farmers and veterinarians in the Kars district of Turkey between 2004 - 2006. In order to achieve this, a total of 407 serum samples of cattle from 27 herds having history of abortions were examined for *Brucella* antibodies by RBPT and SAT. In addition, the sera collected from 246 farmers (130 males and 116 females) and 28 veterinarians in the same district were analysed serologically by RBPT, SAT and ELISA. Of the cattle sera analysed, 134 (32.92%) and 141 (34.64%) were determined as positive by RBPT and SAT, respectively. Thirty-two (13%), 35 (14.22%) and 44 (17.88%) of the farmers' sera were found positive for brucellosis by RBPT, SAT and ELISA, respectively. There was no significant difference

between sexes for*Brucella* seropositivity. Of the 28 sera from veterinarians, 13 (46.42%) were positive by the three serological tests. The high prevalence of brucellosis both in cattle and humans suggests that brucellosis is common in this area. Preventive and control measures should be implemented and pursued more strictly to reduce and/or eradicate brucellosis from the area(**Otlu et al., 2008**).

A cross-sectional epidemiological study was carried out from September 2004 to March 2005 to determine the seroprevalence and identify risk factors for seropositivity of bovine brucellosis in the extensive cattle production systems of Tigray Region. The study populations comprised indigenous breed cattle in the region, and samples were selected by 2-stage cluster sampling. Serum samples collected from816 extensively managed cattle herds above 6 months of age were screened for *Brucella* antibodies by the Rose Bengal Plate Test and reactor sera were further tested by the Complement Fixation Test (CFT). Moreover, information was gathered on individual animal and farm-level risk factors and other farm characteristics using a questionnaire. In this study, the overall seroprevalence of *Brucella* antibodies in the extensively managed cattle was 3.19% based on CFT. The overall herd-level prevalence was 42.31% and the within-herd prevalence varies from 0% to 15.15% based on CFT(**Berhe et al ., 2007**).

Animals sampled were from smallholder dairy farms in Iringa (165 farms) and Tanga (130 farms) regions that were randomly selected from sampling frames of 500 and 3000, respectively. The study also involved seven pastoral traditional herds and one parastatal dairy farm in the Coast region that were purposively selected, based on the willingness of the farmers to participate in the study. The parastatal farm used in the study was located within the neighbourhood of indigenous traditional cattle

herds in the Coast region. A total of 2,187 cattle of various ages (> 2 years), sexes and breeds were thus included in this study which included 762 (34.8%) dairy animals from smallholder dairy farms in Iringa (542) and Tanga (220) regions; 1,350 (61.7%) indigenous traditional cattle from pastoral herds and 75 (3.4%) dairy cattle from one parastatal farm. All animals from pastoral herds were of indigenous (*Bos indicus*) Tanzania Shorthorn Zebu (TSZ) type, while those reared on smallholder or government parastatal farms were crossbred cattle (crosses of *Bos indicus* and *Bos taurus)*

Animals were screened for brucellosis by collecting approximately five ml of blood from the jugular vein of each animal into a plain vacutainer tube. Sera were later separated by centrifugation and immediately frozen at around -20ºC until processed. Whereas serum samples from all indigenous cattle were examined using both Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) in order to assess agreement between the two diagnostic tests, those from crossbred cattle were screened using RBPT alone. Thus, a total of 2,187 sera from cattle were examined by RBPT and 1,349 were subjected to SAT analysis (one indigenous animal had no enough sample for this test). For RBPT, any degree of agglutination was considered as a positive reaction and titre equal to or higher than 1/40 was considered positive for SAT. (**Karimuribo et al., 2007).**

The seroprevalence of *Brucellaspp*. and the possible associated risk factors were estimated for 2,109 adult cows in Monte Negro county, State of Rondônia, Brazil. A questionnaire was completedfor each farm where cattle were sampled. Laboratory tests were Rose-Bengal Agglutination,Standard Tube agglutination, and Mercaptoethanol. The adjusted overall prevalence of *Brucella spp*. seropositive cows for Monte Negro county was 15% and at least 54 herds (63%) were positive.

A logistic regression analysis suggested that the herd size of more than 25 cows and the presence of pigs were significant factors associated with the seropositivity (herd size: OD = 2.8; P=0.02; presence of pigs: OD = 2.5; P = 0.04). Other significant variables associated to the infection, analysed by Chi-Square were the presence of seropositivity cows with theherd that were repeat breeders and birth of weak calves (P<0.05).(**Aguiar et al., 2007**).

Bovine brucellosis, caused by Brucella abortus, is a serious zoonotic disease manifested by reproductive disorders resulting in huge economic losses to dairy farmers. A random survey was conducted to study the epidemiology of brucellosis in Punjab (India) using sampling software Survey Toolbox. Two-stage sampling procedure was adopted; in the first step, villages were selected randomly from sampling frame of all the villages of Punjab followed by selection of owners, and animals in individual farms were identified using random sampling. In all, 32 villages were selected and then 345 animals (approximately 5%) were sampled from these villages. The milk samples collected were screened for brucella antibodies employing ELISA test. The overall apparent prevalence of brucellosis was found to be 18.26% (true prevalence - 17.68%). The prevalence in the central zone of the state was significantl higher, viz. 23.2% (chi square = 11.34, p < 0.01) compared to 14.2% in the sub-mountainous zone and 5.8% in the arid irrigated zone. The disease prevalence was found to be non-significantly higher (chi square 1.029, p = 0.310) in cattle (20.67%) compared to buffaloes (16.41%) and increased with age (chi square = 8.572, p < 0.05) in both species. There was significant association between disease and abortion (chi square

= 22.322, p < 0.01) and maximum abortion cases due to brucellosis werefound in > 6 month of gestation (95.7%). The disease was

significantly associated with the retention of placenta (chi square = 8.477, $p < 0.01$), however there was no significant relationship of the disease with repeat breeding (chi square = 0.044, $p = 0.834$). The results of the study suggested that the accurate epidemiological scenario of the disease may be obtained by employing multistage sampling procedures using milk-based ELISA.(**Aulakh et al., 2008**).

Chapter Two

Materials and Methods

Study Area:

The study was undertaken in Western Equatoria State, which lies between latitudes 11.6-12.8 and longitudes 20.5-23.1 E in rich savanna. The State has area of 40 million feddans with population of 2 million . It is boardered by Democratic Republic of Congo from west, Central Africa Republic from northwest, Eastern Darfur State from north (The Republic of the Sudan), Western Bhar Al Ghazal and Lakes State from northeast, Eastern Equatoria State from the south and Central Equatoria State from the east The state is composed of 10 counties namely – Mundiry East, Mundiry West, Momvolo, Nagiru, Yambio, Maridi, Nazara, Tombora, Saraseibo and Izo

The State is dominated by equatorial climate which is characterized by heavy rains. The average air temperature range between 16.6-24.7, the

mean annual evaporation rate is 7.7 mm/day and daily average humidity of 21 %.

The summer extends from January-March and ends with 10 months rains. The rain fall ranges between 4000-7000 mm per year with peak in June.

The main water sources are river, tributaries, seasonal water (wedian) and ground water. (National Ministry of Agriculture and forestry –Juba 2013)

Study population:

The total animal population in the State is 120,000 heads

Study design and sampling methods:

Data were collected as part of a study on the sero–epidemiology of *brucella* infection in cattle camp in Western Equatoria State. A cross-sectional study was carried out from April to September 2013 to estimate the seroprevalence of bovine brucellosis and to investigate associated risk factors. Multi-stage random sampling was designed based on state, county, payam, herd and animal. Selection between counties, payams, herds and individual animals based on simple random sampling. Four counties selected randomly during the study namely Mundri West, Mundri East, Momvolo and Nagiru

Sampling size:

Sampling size determination:

The sample size was calculated depending on the formula of sample size determination in random sampling (Thrusfield, 1995).

$n= 4*P^{exp}*Q/d^2$

n= required sample size

4=constant

P^{exp}= expected prevalence

$Q= 1-P^{exp}$

d= desired absolute precision

The expected prevalence was considered as 12% in Cairo province, Arab Republic of Egypt (Hegazy et al., 2011).

Desired absolute precision was 5% with 95% confidence interval. The total sample size was calculated as follows.

n=4*0.12*0.88/0.0025=168 animals/samples.

Sampling technique:

Blood samples of 10 ml were aseptically collected using plain tube from cattle through jugular vein puncture. Serum was separated within 12 hours of collection and packaged in a cool box and transported to veterinary central laboratory-Juba , then transported to Khartoum until laboratory tests was performed (RBPT).

Questionnaire survey:

Information of each cattle sample was obtained, this include location, age, sex, breed, body condition and contact with other cattle herds Selected cattle owners were interviewed by using questions. Risk

factors that had possible association with brucellosis among herds were investigated.This risk factors included herd size, age, sex, history of abortion, disposal of foetal membrane, retained placenta, presence of dogs, parity number, source of water, counties.

Diagnostic technique:

RBPT:

All sera samples collected were screened by RBPT using RBPT antigen. Sera samples were kept in refrigerator at 4 c temperature before testing. Sera and antigen were kept at room temperature for half an hour before the test.

The test procedure recommended by (Alton et al., 1975) was followed: 30 µ of RBPT antigen was added to each circle on plate, 30 µ of test serum was mixed thoroughly by wooden application. The plate was shaked for 4 minute and the degree of agglutination reaction were read and recordes as +++ (coarse clumping, clearing), ++ (visible fine agglutination ,+(weak fine agglutination using manifying glass) and in case of positive reactions and 0 (no agglutination) in negative reaction

Statistical analysis:

Data on tested serum and questionnaire results were stored in Microsoft excel spread sheet as database. Statistical analysis was performed using SPSS/ nc, chigaco, IL, USA .

The seroprevalence for animal level was calculated on the basis of RBPT positivity, dividing number of brucella reactors by total number of tested animals. Similarly,herd level prevalence was calculated as the number of herds with at least one positive divided by the total number of herds tested.

Data collected from questionnaire survey was analyzed using descriptive methods. Frequency distribution showed the frequency of occurance of the observation in present data set . Since the present data was categorical the frequency distribution of the variables comprised the frequency occurence of observation in every category.

Crosstabulation was used in 2 x 2tables and related statistics. It illustrated the rate of brucellosis in each category of a risk factor.

Association between outcome variable (status of brucellosis) and it's potential risk factors were first screened in a univariate analysis using Chi-square. Potential risk factors with p-value ≤ 0.25 were considered significant at this level

Significant risk factors in univariate analysis were subjected to multivariate analysis using logistic regression (Odds Ratio/Exp B).

Exp B (Odds Ratio) was used to indicate the strength of association between risk factors and occurrence of disease.

Risk factors with p-value ≤ 0.05 were considered significantly associated with brucellosis.

Chapter Three

Results

In the RBPT, 5 out of the 20 herds studied were seropositive. The overall brucellosis herd prevalence, i.e : at least one positive to RBPT identified in a herd, resulting in 25% herd prevalence (Table 3.1).Then within herd prevalence ranged between 0%-50%.

Table 3.1: Herd prevalence of bovine brucellosis among 20 herds examined by RBPT in Western Equatoria State.

		Frequency	Relative Frequency%	Cumulative Frequency
Valid	positive	5	25	25
	Nagetive	15	75	100
	Total	20	100	

Out of 166 serum samples tested, 21 were positive to Rose Bengal Plate test, resulting in 12.7% individual animal prevalence (Table 3.2).

Table 3.2Prevalence of bovine brucellosis among 166 cattle examined by RBPT in Western Equatoria State:

	Frequency	Relative frequency	Cumulative frequency
Valid positive	21	12.7	12.7
negative	145	87.3	100
Total	166	100	

Prevalence of bovine brucellosis among 166 cattle examined by counties in Western Equatoria state:

The results of this study show the distribution of 166 cattle examined for brucellosis by counties. Forty two serum samples were examined from Mundri West county, 60 from Mundri East county, 39 from Momvolo and 25 from Nagiru county(Table 3.3). Among these counties the prevalence was 25% in Mundri East, followed by 14.5% in Mundri West County, 4% in Momvolo county and 0.0% Nagiru county(Table 3.4). The Chi-square test showed no significant association between infection and counties (p-value : 0.267) (Table 3.5).

Prevalence of bovine brucellosis among 166 cattle examined by age of animals :

A total of 166 cattle of various ages were examined in this study. Table 3.3 shows the age distribution of cattle. Sixty nine were less than and equal 3 years(≤3years) and97 were more than 3 years. The prevalence among the age groups showed that cattle older than 3 years had a prevalence of 59.4% than those less than and equal 3 years 41.6%

(Table 3.4). The Chi-square test showed no significant association between infection and age of animal (p-value : 0.367) (Table 3.5)

Prevalence of bovine brucellosis among 166 cattle examined by sex of animals:

The result of this study showed the distribution of 166 cattle examined for brucellosis by sex. Total number of male examined was 45 while the total number of female examined was 121 (Table 3.3). Among these females , 21 animals was found infected. Rate of infection within females was 18.2%, while among male, no one was found infected. The rate of infection within male was 0.0% (Table 3.4). The Chi-square test showed no significant association between infection and sex of animal (p-value : 0.311) (Table 3.5).

Prevalence of bovine brucellosis among 166 cattle examined by body condition of animals :

Body condition of animals and presence of brucellosis have been investigated. One hundred and twenty three(74.2%) of cattle were found to be in good condition (Table 3.3) and the rate of infection in these was 12.2%, while forty five (27.2%) cattle were found to be in poor condition with infection rate 15.6% (Table 3.3). The chi-square test showed significant association between the infection and body condition (p-value : 0.200) (table 3.5)

Prevalence of bovine brucellosis among 166 cattle examined by number of parity :

The result of this study showed the distribution of bovine brucellosis infection by number of parity (Table 3.3). Eighty five cattle were null porous, 14 cattle were one parity, 45 cattle were more than one

parity. The prevalence among the number showed that cattle with more than one parity had prevalence 27.1%, follow 8.4% and null porous 51.2% (Table 3.4). The Chi-square test showed no significant association between the infection and number of parity (p-value 0.153) (Table 3.5)

Prevalence of bovine brucellosis among 166 cattle examined by presence of dogs:

The result of this study showed the distribution of bovine brucellosis infection by the presence of dogs in the camp. Seventy five (45.2%) cattle had no dogs in the camps and 69(41.6%) cattle had dogs in the camp (Table 3.3). the rate of infection is higher in the absence of the dogs (22.7%) compared to rate of infection (19.1%). The Chi-square test showed no significant association between the infection and the presence of dogs (p-value 0.356)(Table3.5)

Prevalence of bovine brucellosis among 166 cattle examined by disposal of placenta:

The result of this study showed the distribution of bovine brucellosis infection by disposal of placenta. One hundred and thirty eight (83.1%) of cattle had no foetal membrane disposal 28 (16.9 %) cattle had foetal membrane disposal (Table 3.3), (Table 3.4) determined that the rate of infection is high when foetal membrane is disposed. 19 (50 %) cases were reported when placenta were disposed, compared with three (13.8%) cases when the placenta were not disposed. The chi-square test showed significant association between the rate of infection and disposal of placenta (p-value 0.244) (Table 3.5)

Prevalence of bovine brucellosis among 166 cattle examined by herd size :

The result of this study showed the distribution of bovine brucellosis infection by herd size (Table 3.3). Thirty six(21.7%) of cattle had small herd size and 130 (21.7%) of cattle had large herd size (table3), determine the rate of infection with the herd size. Zero (0.0%) cases were reported when the herd size were small, compared with 22 (16.9%) cases when the herd size were large. The Chi-square test showed significant association between the rate of infection and herd size (p-value 0.201), (Table 3.5)

Prevalenceof bovine brucellosis among 166 cattle examined by history of abortion:

The result of this study showed the distribution of bovine brucellosis infection by history of abortion (Table 3.3), 118 (71.1%) had no history of abortion and 48 (28.9%) cattle had history of abortion. Seventeen (10.2%) cases reported when there was history of abortion compaired with 5 (3.o %) cases reported when was no history of abortion (table 3). The Chi-square test showed significant association between the rate of infection and history of abortion (p-value 0.131), (Table3.5)

Prevalenceof bovine brucellosis among 166 cattle examined by history of retain placenta:

The result of this study showed the distribution of bovine brucellosis infection by history of retain placenta, 149 (89.8%) of cattle had no retain placenta and 17 (10.2%) cattle had history of retain placenta, compared with 71 (4.7%) cases when were no history of retain placenta (Table 3.3). The Chi-square test showed that there was

significant association between the rate of infection and history of abortion (p-value 0.115), (Table 3.5)

The Chi-square univariate analysis revealed six variable with p-value ≤ 0.20 were statistically significant (table 4). These 6 factors were entered to multivariate logistic regression, all factors in multivariate analysis were statistically not significant and had p-value more than > 0.05 (Table 3.5)

Table 3.3 :Summary frequency table for distribution of 166 serum samples examined by RBPT according to potential

Risk Factors :

Risk Factor	Frequency	Relatives frequency %	Cumulative frequency%
Counties : Mundri West	42	25.3	25.3
Mundri East	60	36.1	61.4
Momvolo	39	23.5	86.7
Nagiru	25	15.1	100
Total	166	100.0	
Age : ≥ 3Years	69	45.2	45.2
˂ 3 Years	97	54.8	100
Total	166	100	
Sex : Female	121	81.8	81.8

Male	45	18.2	100
Total	166	100	
Body condition :Good	123	74.2	74.2
Poor	43	25.8	100
Total	166	100	
Parity number:Null	85	51.2	51.2
One parity	26	15.7	66.9
˃ One parity	55	33.1	100
Total	166	100	
Presence of dog : Yes	91	54.8	54.8
No	75	45.2	100
Total	166	100	
Disposal of placenta : Yes	28	16.9	16.9
No	138	83.1	100
Total	166	100	
Herd size : small≤20	36	21.7	21.7
Large ˃ 20	130	78.3	100
Total	166	100	
History of Abortion: Yes	48	28.9	28.9
No	118	71.1	100
Total	166	100	
History of Retained placenta: Yes	17	10.2	10.2
No	149	89.9	100

Table 3.4 :Summary cross- tabulation for prevalence of brucellosis with potential risk factors .

Risk Factors		No-tested	No-positive	Percent
County :	Mundri West	42	6	14.2
	Mundri East	60	14	25.0
	Momvolo	39	—	—
	Nagiru	25	1	4
Total		166		
Age : ≥ 3Years		69	4	5.8
< 3 Years		75	17	25.3
Total		166		
Sex :	Female	121	21	18.2
	Male	45	—	—
Total		166		
Body condition :	Good	123	16	13.8
	Poor	43	5	11.6
Total		166		
Parity number:	Null	85	—	—
	One parity	26	9	34.6
	> one parity	25	12	23.6
Total		166		
Presence of dog :	Yes	91	6	6.6
	No	75	15	21.3

Total	166		
Disposal of placenta : Yes	28	5	17.9
No	138	16	12.3
Total	166		
Herd size : small ≤ 20	36	—	—
Large ˃ 20	13	21	16.9
Total	166		
History of Abortion: Yes	48	13	29.2
No	118	8	6.8
Total	166		
History of Retain placenta: Yes	17	10	58.8
No	149	11	8.1
Total	166		

Table 3.5 : Summary of univariate analysis for potential risk factor of bovine brucellosis in 166 cattle examined in Western Equatoria State using the Chi-square test :

Risk Factors	No tested	No- +ve (%)	df	X^2	p-value
1- Sex : Female	121	21(18.2)	1	_	_
Male	45	0.0%	_	0.492	0.311

2- Age :≥ 3Years	69	0(5.8)	1	0.381	0.367
˂ 3 Years	75	21(25.3)			
3- Body condition : Good	123	16(13.8)	1	0.511	0.200
Poor	43	5(11.6)			
4- Parity number: Null	85	8(34.6)	2	0.637	0.153
One parity	26	11(23.6)			
˃ One parity	55	2(27.5)			
5- Presence of dog : Yes	91	5(6.6)	1	0.521	0.356
No	75	16(21.3)			
6-Disposal of placenta: Yes	28	4(17.9)	1	1.348	0.244
No	138	17(12.3)			
7- Herd size : small ≤ 20	36	—	1	1.863	0.201
Large ˃ 20	130	21(16.9)			
8-History of Abortion: Yes	48	14(29.2)	1	0.825	0.131
No	118	7(5.8)			
9-History of Retained placenta:Yes	17	10(58.8)	1	1.403	0.115
No	149	11(25.0)			
10- County : Mundri West	42	5(14.2)	3	1.682	0.267
Mundri East	60	15(25.0)			
Momvolo	39	—			
Nagiru	25	1(4)			

Table 3.6 : **Multivariate analysis for potential risk factor of bovine brucellosis in 166 cattle examined in Western Equatoria State using logistic Regression(Odds Ratio):**

Risk Factors	No-tested	No-+ve(%)	Exp(B)	95%c.I for Exp(B)	p-value
Body condition :Good	123	17(13.8)	0.199	0.986_0.410	0.371
Poor	43	4(11.6)			
Parity number: Null	85	__	1.419 E	0.322	0.481
One parity	26	9(34.6)			
˃ one parity	55	12(23..6)			
Herd size : small ≤ 20	36	_	7.787 E7	0.358	0.362
Large ˃ 20	130	21(16.9)			
History of Abortion: Yes	48	13(29.2)	0.214	0.548	0.283
No	118	7(6.8)			
History of Retain placentaYes	17	10(58.8)	0.386	0.210	
No	149	11(8.1)			0.526
Disposal of Placenta Yes	28	4(17.9)	0.614	0.407	0.382

No	138	17(12.3)

Chapter Four

Discussion:

Brucellosis is considered as an important cause of productive losses in cattle. In addition, it is a zoonosis thoroughly diffused all over the world (Radiostits et al.,2000). This study showed a seroprevalence of brucellosis in cattle in Western Equatoria State, South Sudan as 12.7%. This result was in agreement with another result carried out by Dinka and Chala,(2009) in

Oromia regional state , Ethiopia , with a prevalence rate of 11.2% .

The prevalence reported in this study was higher than that reported by Kaoud et al., 2010 in Egypt which was 2.16% herd prevalence, in Bangladesh which was 2.13% herd prevalence, (Rahman et al, 2011) , Mohammed et al, (2011), in Sarab city, Iran which was 3.66% herd prevalence .

These western Equatoria State seroprevalence was lower than that reported by Angara et al.,(2004) in Kuku diary Scheme, Khartoum North, Sudan which was 31.0%. The result also was lower than that reported by Berhe et al., (2007) in extensive cattle production system of Tigray Region of Ethiopia , where they obtained 42.3% herd prevalence.

The present result is lower than that reported by Megersa et al., (2011) in Southern and Eastern Ethiopia where he obtained 31.0% herd prevalence . And also lower than that reported by Aguiar et al., (2007) in Western Amazon, Brazil, with 63.0% herd prevalence rate.

In addition to investigating the prevalence, this study was conducted with the objective of identifying potential risk factors associated with brucellosis seroprevalence in cattle in Western Equatoria State, South Sudan. In this study history of abortion, waste disposal, parity number, age, history of retained placenta were identified as the risk factors associated with seropositivity to Brucella antigen at the univariate analysis using Chi-square test(P-value≤0.25). No one of these risk factors was significant in the multivariate analysis using logistic regression (P-value˃0.05).

Bovine Brucellosis was detected in three counties of Western Equatoria State with high prevalence in Mundri East following in descending order Mundri West, Momvolo

and Nagiru. This variation in the prevalence could be due to difference in agro- ecological and management system. Mundri East had huge number of animals of different species and rich with agricultural lands which made it the major area for animal rearing. Most animals in this county graze in common pasture and around water source and some of these animal may be carriers of *brucella* infection and these could be a potential problem for spread of the disease while in Mundri West, Momvolo and Nagiru Counties most of the animal kept in the house hold. This finding is similar to the study of Berto et al.,(2010) which reptorted a relatively higher prevalence in zone dominated by free range management system compared to confinement zone.

The study also revealed that there was no significant difference between male and female cattle although a higher prevalence was found in female than in males. A similar finding was reported by Ashensfi et al.,(2007) and shagrie et al, (2011) but opposite to that was reported by Islam et al., (2009) . The higher prevalence in females could be attributed to the fact that female sex hormones and erythritol stimulate the growth and multiplication of *brucella* (Radostits et al., 2000).

A higher seroprevalence against brucella was detected in adults than in youngers cattle. This was also recorded by Ferede et al.,(2011). However it has been recorded that the susceptibility to brucellosis appear to be more commonly associated wih sexual maturity. Sexual mature and pregnant animals are more prone to *brucella*infection (Radostits et al, 2000). However the association between brucellosis and sexual maturity is not statistically significant in our study.

Herd size was found to be statistically significant with brucellosis seropositivity in this study in the univariate analysis. Similar finding had been documented by Coelho

et al., (2004) and Al- Majali et al.,(2005). This result could be attributed to the fact that large herds tend to be raised under extensive management system, which may increase the possibility of transmission of the disease through direct contact.

The result of this study showed a significant relationship between brucellosis and previous history of abortion and retained placenta. This result is in agreement with Islam et al., (2009), and Ashagrie et al.,(2011). This could be explained by the fact that the infection localized in the placenta and leads to development of placentits with subsequent abortion and after abortion uterine infection persist up to 5months, (Radostits et al., 2000).

The current study encounter a high seropositivity within animals depends on the pasture as source of feed for cattle compared to other sources. This could be due to high exposure of the cattle camps to contamination by infected material and discharges from infected cattle which persist for longer period in the environment.

No significant difference was observed by using common pasture, contact with other animals and presence of dogs, also no statistical significant difference were found for vaccination and using separate pens for parturition, because all cattle keepers agreed on the absence of these practices on their camps.

A relatively higher infection rate was recorded in cattle keepers shared males with other camps. This could be explained by the fact that infected males may discharges semen containing *brucella*organism and it is likely to transmit the infection to the females. Similar finding was recorded by Lithg- Pereira et al,(1999).

Also, higher infection rate was recorded in cattle whose keepers had dogs reared with their camps. Similar finding was recorded by Samadi et al., (2010). This could be

explained by the fact that keepers who provided aborted fetuses or infected placental membrane to the dogs provide possible route for disease transmission from cattle to dog and later from dog to cattle. However this risk factors was not found statistically significant in this study.

In spite that the factors of vaccination and using separate pens for parturition were not computed, but we cannot ignore the fact that these factors could play a major role in the disease prevention and control. Some studies revealed statistically significant association between vaccination and *brucella* prevalence (Samadi et al., 2010), (Lithg-pereira et al, 1999), and (Al-Majali et al., 2005).

In our study, the result revealed that cattle that raised by keepers who did not dispose fetal membranes recorded higher prevalence than those who dispose fetal membrane. However result showed no statistically significant association between brucellosis and dispose of fetal membrane. This could be due to the fact that infected cattle that abort or give birth normally discharge large numbers of *brucella* in their uterine exudates and placenta, so the fetal membranes could be a major source of infection (Radostits et al., 2000).

Conclusion

The study concluded that Western Equatoria State should be considered as endemic with bovine brucellosis which must require control strategies. Reaction due to vaccine titres was excluded, because there was no clear and documented history of previous vaccination in the survey.

Brucellosis situation in Western Equatoria State should be tackled seriously considering the zoonotic nature of the disease, the heavily populated area supplied by milk produced from cattle camps and the feeding habit of in-contact people who used to drink raw milk.

Recommendations

The study recommended formulation of long term plan to control the disease in the South Sudan. Further study of the disease in Western Equatoria State is recommended, providing awareness of zoonotic disease especially brucellosis to butchers and livestock keepers, providing preventive materials, such as gloves to butchers and livestock keepers, testing exotic animals before entering the country.

It is imperative to investigate the disease in human, specially among animal handlers and particularly pastoralist who indulge in the practice of manipulation obstetrical disorders of cow without protective gloves.

Control programs could make a useful contribution towards preventing brucellosis in cattle and decreasing losses in the livestock Population . More attention should be paid towards implementing a proper control program

for brucellosis and more efforts should be directed towards improving the animal health biosecurity program. In addition, controlling brucellosis in calves (mainly by strain -19 vaccination) will reduce the prevalence of this disease in cattle.

Control progress should be monitored serologically and evaluated epidemiologically, veterinary extension should play a major role to guarantee the application of sanitary procedures and measures in rearing raising and breeding place places and education of personnel and dissemination of awareness as well as veterinary public health culture through various multimedia.

Improvements are needed in hygienic practices to decrease the chances of disease spread. Awareness should be promoted among cattle keepers in the area as to the importance of the hygienic disposal of aborted Fetuses.

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Appendix 1

Frequency table for distribution of 166 cattle examined for brucellosis by RBPT in the Western Equatoria State according to potential risk factors:

Appendix 1.1: County

	Frequency	Percent	Cumulative %
valid			

Mundri East	60	36.1	36.1
Mundri West	42	25.3	61.4
Momvolo	39	23.5	84.9
Nagiru	25	15.1	100
Total	166	100	

Appendix 1.2: Age

	Frequency	Percent	Cumulative %
valid			
3 years	69	41.6	41.6
3 years	97	58.4	100
Total	166	100	

Appendix 1.3: Sex

	frequency	Percent	Cumulative %
Valid			
Male	45	27.1	27.1
	121	72.9	100
Female			
Total	166	100	

Appendix 1.4: Body Condition:

	frequency	Percent	cumulative %

Valid			
Poor	43	25.9	25.9
Good	123	74.1	100
Total	166	100	

Appendix 1.5: Parity Number:

	frequency	Percent	cumulative %
Valid			
One parity	26	15.7	15.7
One parity	55	33.1	48.8
Null	85	51.2	100
Total	166	100	

Appendix 1.6: presence of Dogs:

	frequency	Percent	Cumulative %
Valid			
No	75	45.2	45.2
Yes	91	54.8	100
Total	166	100	

Appendix 1.7: Herd Size:

		frequency	Percent	Cumulative %
Valid				
	20	36	21.7	21.7
	20	130	78.3	100
	Total	166	100	

Appendix 1.8: Disposal of Placenta:

		frequency	Percent	Cumulative %
Valid				
	Yes	28	16.9	16.9
	No	138	83.1	100
	Total	166	100	

Appendix 1.9: History of Retain placenta:

		frequency	Percent	Cumulative %
Valid				
	Yes	17	10.2	10.2
	No	149	89.8	100
	Total	166	100	

Appendix 1.10: History of Abortion:

		frequency	Percent	Cumulative %
Valid				
	Yes	48	28.9	28.9
	No	118	71.1	100
	Total	166	100	

Appendix 2

Cross tabulation of the seroprevalence of Brucellosis in 166 cattle examined by RBPT Western Equatoria State according to potential risk factors:

Appendix 2.1: Seroprevalence * Age

	Age		
	˃3 year	˂3 years	
RBPT	69	76	145
	69/69*100	76/97*100	87.3%
Negative	100%	78.4%	
	0	21	21
Positive	o/69*100	21/97*100	12.7%
	0.0%	21.6%	
Total	69	97	166
	100%	100	100%

Appendix 2.2: Seroprevalence * Age

		Sex	
	Male	Female	Total
RBPT	45	100	145
	45/45*100	100/121*100	87.3
	100%	82.6%	
Negative			

Positive	0	21	21
	0.0%	21/121*100	12.7
		17.4%	
Total	45	121	166
	100%	100%	100%

Appendix 2.3: Seroprevalence * Body Condition

	Body Condition		
	Good	Poor	Total
RBPT	105	40	145
Negative	105/123*100	40/43*100	87.3%
	85.4%	93.0%	
Positive	18	3	21
	18/123*100	3/43*100	12.7%
	14.6%	7.0%	
Total	123	43	166
	100%	100%	100%

Appendix 2.4: Seroprevalence * Number of Parity:

	Number of parity			
	Null	One Parity	˃One parity	Total
RBPT	85	21	49	155
	85/85*100	21/26*100	49/55*100	87.3%
Negative	100%	80.8	89.0%	
Positive	0	5	16	21
	0.0%	5/26*100	16/55*100	12.7%
		19.2	11.0%	
Total	85	26	55	166
	100%	100%	100%	100%

Appendix 2.5: Seroprevalence * Presence of Dogs

	Presence of Dogs in Camps		
	NO	Yes	Total
RBPT	67	77	144

		77/91*100	
	67/75*100	84.6%	87.3%
Negative	89.3%	13	
	8	14/91*100	21
Positive	8/75*100	15.4%	12.7
	10.7%	91	
	75	100%	166
Total	100%		100%

Appendix 2.6:Seroprevalence * Disposal of Placenta:

	Placenta Disposal		
	Yes	No	Total
RBPT	11	134	145
	11/28*100	134/138*100	
Negative	39.3%	97.1	87.3%
		4	
	17	4/138*100	21
Positive	17/28*100	3.9	12.7%
	60.7%		
	28	138	166

Total	100%	100%	100%

Appendix 2.7:Seroprevalence *Herd Size:

	Herd Size		
	Small 20	Large 20	Total
RBPT	36	109	145
	36/36*100	109/130	87.3%
Negative	100%	83.9	
Positive	0	21	21
	0.0%	21/130*100	12.7%
		16.1%	
Total	36	130	166
	100%	100%	100%

Appendix 2.8: Seroprevalence * History of Retained Placenta:

	History of Retain Placenta		
	No	Yes	Total
RBPT	132	13	145
Negative	132/146*100	13/20*100	87.3%
	90.4%	65.0%	
Positive	14	7	21
	14/146*100	7/30*100	12.7%
Total	9.6%	35.0%	
	146	20	166
	100%	100%	100%

Appendix 3

Chi- Square test

Univariate analysis for the association of the seroprevalence of brucellosis in 166 cattle examined by RBPT in Western Equatoria State with risk factors:

Appendix 3.1:Association between bovine brucellosis infection and County

	Value	df	Significant
Pearson chi square	3.145	3	0.293
Likelihood Ratio	4.02	3	0.30
Linear by Linear Association	1.56	3	0.34
N of valid cases	166		

Appendix 3.2:Association between bovine brucellosis infection and age

	Value	df	Significant
Pearson chi square	1.245	1	0.31
Likelihood ratio	2.41	1	0.28
Linear by Linear Association	0.26	1	0.35
N of valid cases	166		

Appendix 3.3: Association between brucellosis infection and sex:

	Value	df	Significant
Pearson chi square	0.421	1	0.37
	1.25	1	0.39
Likelihood ratio	0.36	1	0.47
Linear by Linear Association			

Appendix 3.4: Association between bovine brucellosis infection and body condition

	Value	df	Significant
Pearson chi square	1.02	1	0.45
Likelihood Ratio	1.41	1	0.52
Linear y Linear Association	0.01	1	0.35

Appedix 3.4: Association between brucellosis infection and number of parity

	Value	df	Significant
Pearson chi square	0.61	2	0.64
Likelihood			

Ratio	0.56	2	0.61
Linear by Linear Association	0.35	1	0.48

Appendix 3.5: Association between brucellosis infection and presence of dogs:

	Value	df	Significant
Pearson chi square	0.14	1	0.41
Likelihood Ratio	0.19	1	0.61
Linear by Linear Association	0.10	1	0.42

Appendix 3.6: Association between brucellosis infection and disposal of placenta

	Value	df	Significant
Pearson chi square	0.71	1	0.33
Likelihood Ratio	0.64	1	0.31

Linear by Linear Association	0.62	1	0.36

Appendix 3.7:Association between brucellosis infection and herd size:

	Value	df	Significant
Pearson chi square	0.17	1	0.29
Likelihood Ratio	0.15	1	0.31
Linear by Linear Association	0.10	1	0.37

Appendix 3.8: Association between brucellosis infection and waste disposal

	Value	df	Significant
Pearson chi square	0.41	1	0.35
Likelihood Ratio	0.46	1	0.31
Linear by Linear Association	0.47	1	0.38

Appendix 3.9:Association between brucellosis infection and history of retained placenta

	Value	df	Significant
Pearson chi square	1.12	1	0.28
Likelihood Ratio	1.64	1	0.34
Linear by Linear Association	1.25	1	0.39