Chapter One

Introduction

1.1 Background:

Brucellosis is an infectious disease of domestic and wild animals, with serious zoonotic implication in humans. The disease is an important public health problem in many parts of the world. Cattle, goats, pigs, sheep, horses, and dogs play an important role in the transmission of brucellosis to man (Akbarmehr and Ghiyamirad, 2011).

Brucellosis, also known as “undulant fever, Mediterranean fever” or “Malta fever” 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 of 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, 2006).

Brucellosis is defined as a contagious bacterial disease primarily of Ruminants, characterized by inflammation of the genital organs and fetal membrane, abortion, sterility, and formation of localized lesions in the Lymphatic system and joints (Cadmus et al., 2010).

Brucellosis is a contagious infectious disease caused by bacterial species of the genus *Brucella*. 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 freefrom the agent, (OIE, 2009).

Brucellosis is the most important zoonosis in terms of human suffering and is a true zoonosis in that almost all human cases are acquired from animals , in particular goats and sheep . In Egypt, brucellosis is still remaining one of them major diseaseproblems that affect animal industry as well as human health and is still an endemic serious disease among domestic animals and humans in spite of attempts that were implemented to control the disease through bilateral projects with some agencies or international organization. (Kaoud1et al., 2010).

Brucella melitensis occurs naturally in sheep and goats and is highly pathogenic for humans, causing one of the most serious zoonosis in the world. The disease is responsible for considerable economic losses to the small ruminant industry (Benkirane 2006, OIE 2009). Sheep and goats brucellosis is endemic in most countries of the Mediterranean basin, the Middle East and Central Asia (Al-

Majaliet al 2005), Latin America, and parts of Africa (Benkirane et al 2006).

The first report of brucellosis in Portugal is from1873. An eradication program was initiated in Portugal, in 1990, in small ruminants, with the financial support of the European Commission. This programwas based on test and slaughter policy, using Rose Bengal Test (RBT) and Complement Fixation Test (CFT) and the farmers received compensation for the slaughtered animals. A new program of control and eradication started in Portugal with flock vaccination during 2001 - 2004 with the live *Brucell amelitensi s*reversion 1 strain vaccine (Rev. 1 vaccine, conjunctival route and dose of 1x109), and continued the following years with vaccination of young replacements (Netoand Vaz 2002).

Traditionally, brucellosis diagnosis was based in the detection of circulatingAntibodies followed by bacteria isolation of the microorganisms (Cassataro *et al* 2004). Bacteriological diagnosis has lack of sensitivity, and is not a practical and reliable means for diagnosis in large-scale programs (Cassataro *et al* 2004). These limitations make serology the most useful epidemiological tool for laboratory diagnosis of *Brucella* infection (Erdenebaater *et a l*2004).

There are six classical *Brucella* species, which differ from one another in their choice of animal hosts. Other differences observed include biochemical characteristics, culture appearance and the amount or number of the main antigens they possess (Stack and Macmillan, 2000). The major species are *B. abortus* which infects cattle; *B. melitensi s*affecting goats and sheep; *B. suis* affecting pigs; *B. canis* which infects dogs*; B. ovis* which infects sheep and *B. neotome* which infects desert rats. *B. microfti* has been isolated from soil and mice (Cloeckaert *et al*., 2002).

1.2 Justification:

The disease can generally cause significant loss of productivity through abortion , prolong calving ,kidding or lambing interval ,low herd fertility , and comparatively low milk production in farm animals. The disease could seriously impair socio-economic development for livestock owners ,which represent a vulnerable sector in rural population in general and pastoral communities in

particular.it has a significant public health impact for people who are in direct contact with animals, low awareness and poor hygienic condition which favor for infection (Megersa , 2008).

1.3 Objectives:

The objectives of this study were:

1/ to determine the prevalence of caprine brucellosis in Elgenaina State.

2/ to investigate the risk factors associated with caprine brucellosis.

Chapter two

Literature review

2.1 General overview of Brucellosis

In 1860, Marston provided the first modern clinical description of brucellosis and named it Mediterranean gastric remittent fever, while Bruce and Carrauna–Seciuna of Malta in 1887 demonstrated the etiological role of *Brucella melitensis* (Rust, 2006). This organism was first isolated from the brain of a goat in 1897 by Hughes who published a classic description of this illness (Amato *et al*., 1995). His term “undulant fever” became the most widely accepted clinical description until “brucellosis” became the most commonly used name. In 1924, Lemaire first isolated *Brucella melitensis* from the spinal fluid of a goat (Rust, 2006).

2.2 Etiology*:*

The etiological agent of brucellosis is a bacterium of the genus *Brucella* and the species are *B. melitensis, B. abortus, B. suis, B.ovis, B. canis, B. neotome, B. microfti*and recently isolated from marine animals *B. maris* (Cloeckaert*et al*., 2001)

2.3 Morphology of *Brucella:*

Brucellosis is caused by a group of gram-negative cocco-bacilli belonging to the genus *Brucella* (Fig.1). These bacteria are essentially pathogens of cattle, goats, sheep and pigs (Coghlan, 1995). *Brucella* species are aerobic with the exception of *Brucella abortus*, which requires 5-10% carbon dioxide for growth (Alton *et al*., 1988). All *Brucella*

strains grow well in media enriched with animal serum and glucose at an optimum temperature of 37°C (Alton *et al*., 1988). *Brucella* occurs singly, in groups or short chains and is nonmotile, non-capsulated and non-sporing (Anonymous, 1997). On solid medium they aresmooth, moist, translucent and glistering colonies which may take several days to appear the organisms tend to mutate phenotypically forming rough colonies (Anonymous,1997).

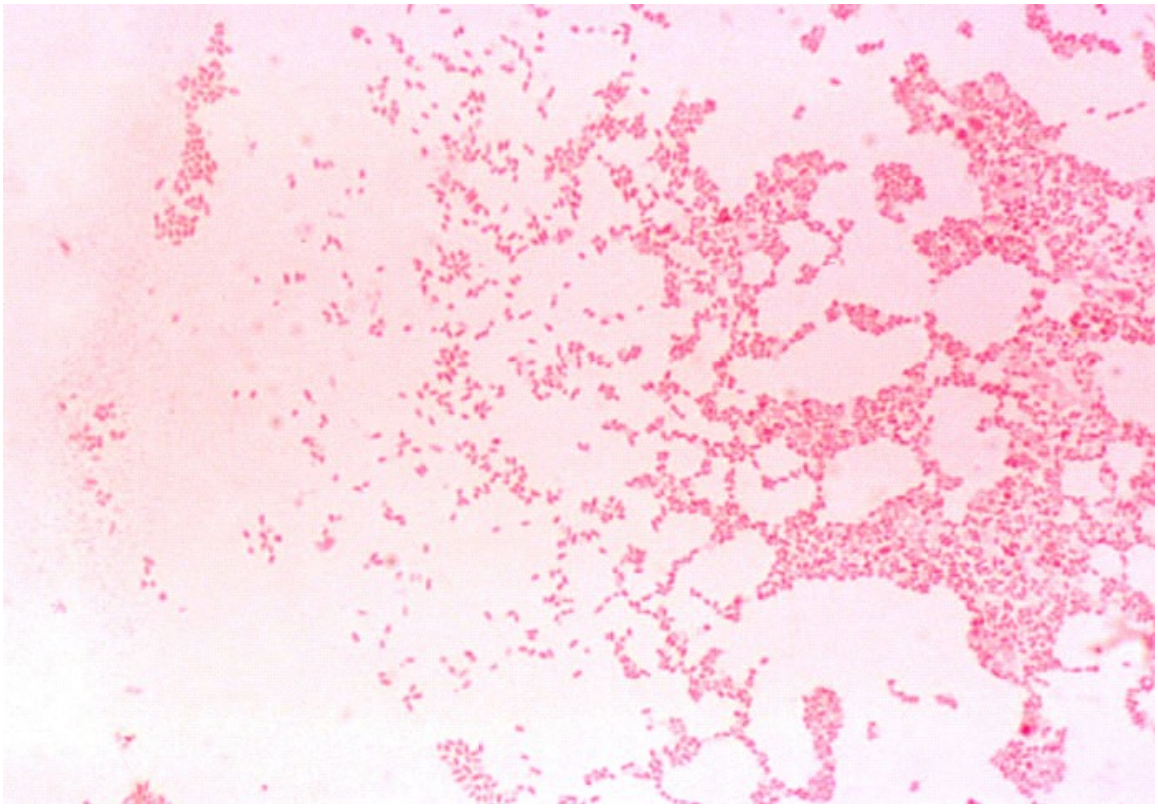

Figure 1: A Gram-stain of *Brucella abortus* showing the Gram-negative coccobacillary shape. Source: Centers for

Disease Control, Public Health Image Library number 1937. (Anonymous, 2002).

2.4 Virulence and Pathogenicity:

The establishment and outcome of infection with *brucella* depend on the Number of infecting organisms and their virulence and also on host susceptibility *Brucella*, which lack the major outer-membrane lipopolysaccharide, produce rough colonies and are less virulent than thosederived from smooth colonies. Although smooth and rough organisms canenter host cells, rough forms are usually eliminated unlike smooth formswhich may persist and multiply (Quinn *etal*.1999).Virulent when engulfed by phagocytes on mucous membranes, aretransported to regional lymph nodes. *Brucellas* 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 *brucella* to survive within macrophages are not fully elucidated. Various stress proteins are thought to allow the organisms to adapt to harsh conditions encountered within macrophages (Quinn *et al.,* 1999).

Brucella can enter mammalian hosts throughskin abrasions or cuts, the conjunctiva, the respiratory tract, and thegastrointestinal 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 killthem, and are also phagocytosed by macrophages. Bacteria transported inmacrophages, which travel to lymphoid tissue draining the infection site,may eventually localize in lymph nodes, liver, spleen, mammary glands,joints, kidneys, and bone marrow, (Purcell *et al,* 1997.)In macrophages, *brucella e*inhibit fusion of phagosomes and lysosomes, and replicate within compare ments that contain components ofendoplasmic reticulum via a process facilitated by the type IV secretionsystem. If unchecked by macrophage microbicidal mechanisms, the bacteria destroy their host cells and infect additional cells. *Brucella* can also replicate extracellularly in host tissues, (Purcell *et al,* 1997).

2.5 Transmission:

Generally, transmission occurs in the same way in sheep and goats as incattle, materials excreted from the female genital tract forming the mainsupply of organisms for transmission to other animals and man. Therefore, in most circumstances, the primary route of dissemination of *Brucella* is the placenta, foetal fluids and vaginal discharges expelled by infected ewes afterabortion or full-term

parturition. Very large numbers of organisms are shed at the time of parturition or abortion. In goats, excretion of the organismsfrom the vagina is prolonged and copious (2 to 3 months generally). In sheepexcretion is generally less prolonged, usually ceasing within 3 weeks afterabortion or a full-term parturition. Shedding of *Brucella* is also common in udder secretions and semen, and *Brucella* may be isolated from varioustissues, such as lymph nodes from the head and those associated withreproduction, and sometimes from arthritic lesions (Alton *et al.,* 1988)

2.6 Pathogenicity

Brucella are facultative intracellular parasites of the reticuloendothelial system. The virulence of *Brucella* varies considerably according to species, strain and the size of infecting inoculum. 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 parasite-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), while in other animals the symptoms are variable. Pathogenically, *Br. Melitensis* infection in sheep and goats is similar to *B.aborts* infection in cattle. Nevertheless,

differences are significant, and each species of *Brucella* causes a different disease (OIE Manual, 1996). In man it is caused by direct or indirect contact with infected animals and the infection usually cause severe or chronic illness.

2.7 Pathogenesis:

2.7.1 Pathogenesis of Brucellosis in animals:

Pathogenically, *B.melitensis* infection in sheep and goats is similar to *Brucella* infection in cattle. Nevertheless, differences are significant, and each species of *Burcella* causes a different disease (OIE Manual, 1996).

In animals, chronic *Brucella* infections have been associated with survival mechanisms, namely initial survival and dissemination of the organisms (Riley and Robertson, 1984). Primarily the virulent factor is the lipopolysaccharide (LPS) which protects the organism from complement-mediated lysis, enhancing intracellular survival (Rege *et al*., 2006). The dissemination is based on the inhibition of primary degranulation and oxidative bursts in polymorphneutrophils, thereby preventing phagolysosomal fusion (Riley andRobertson, 1984). This is morelikely to occur in cases where the initial antibiotic treatment of brucellosis was inadequate. Since chronic brucellosis does not develop in all untreated individuals, other host factors may be playing a role in susceptibility to chronic infection. For example, certain individuals may be

more vulnerable than others to development of a chronic state of infection because they have lower than average immune competence specific for *Brucella* species. (Latimer *et al*., 1992)

2.7.2 Pathogenesis of brucellosis in man:

Brucellosis in human is transmitted through ingestion of contaminated unpasteurized milk or other animal food products such as improperly cooked meat (Rust, 2006). From thegastrointestinal tract, the bacteria pass through the mucosa into the blood stream and thecirculatory system. However, transmission may occur through cuts, abrasions, inhalation and direct contact with mucous membranes (Rust, 2006). The organism quickly becomesan intracellular pathogen, colonizing the lymphatic system (i.e. lymph nodes, spleen, andbone marrow) as well as the liver (Enright, 1990). The bacteria seek cells that are capable of providing the nutrient erythritol, hence their predilection towards genital tracts ofanimals (Rust, 2006). Reticular endothelial cells, particularly themacrophages are also preferred in animals and are the chief site of the infection inhumans (McDermott *et al*., 1994 Rust, 2006). The organisms often enter macrophages using host micro-filaments, where they are protected from the various defense mechanisms of the immune system (Finlay and Falkow, 1989). The protection mechanism involves the

capacity of the inter nalised bacteria to evade the phagosome –lysosome fusion pathway. In advanced stages, in men, frequently there may be orchitis (Villafane*et al*., 1948).

2.8 Animal brucellosis:

Brucella infections are widely distributed in domesticated animals especially in thedeveloping World (Corbel, 1997; Godfroid, 2002). Cattle infections are commonly caused by *Brucella abortus* (Corbel, 1997). In cases where cattle come in contact with infected pigs or goats, *Brucella suis* and *Brucella melitensis* infections may take place (Corbel, 1997; Godfroid, 2002). However the two strains usually cause less severedisease in cattle. Infection is most commonly through ingestion, contact with foetal and placental contents while *Brucella abortus* can also be transmitted through coitus (Foster and Smith, 2008). Young cows are less susceptible compared to mature or older animals which tend to be sexually active since brucellosis is considered to be more of a sexually transmitted disease among animals (Parker, 2007). Unborn calves are usually aborted at about seven months and in case of birth, they are weak and die shortly afterwards (Corbel, 1997). In terms of milk production, a severe drop is experienced as a result of infection in the herd (Bandara and Mahipale, 2002). There are large swellings in the joints of limbs called hygromas in

infected cows (Anon., 2002). Brucellosis mainly affects sexual organs with serious results of endometritis and epididymitis (Bandara and Mahipale, 2002). Bulls may exhibit sterility and orchitis. The infected herd may also exhibit disabilities such as discospondylitis, bursitis or arthritis (McDermott *et al*., 1994; Traboulsi *et al*., 2007). Pigs are affected most commonly by *B. suis* (Godfroid, 2002). However, pigs may also be affected by *B abortus in* cases where they come in contact with infected cattle (Stuart *et al.,* 1987). Sexual contact and ingestion may be the modes of transmission (Godfroid, 2002). In sheep and goats, *B.melitensis*is the classical species affecting females of both animal species (Diaz-Aparicho*et al*., 1994). In cases where infected cattle or pigs come in contact with small ruminants, infections of *B.abortus* and *B.suis* can occur (Stuart *et al.,* 1987). *B.melitensis* infections are acquired primarily by ingestion (Alton, 1990). Abortion and mastitis usually occur in infected goats (Corbel, 1997). Dog brucellosis is most commonly caused by *B.canis* (Foster and Smith, 2008).

However infections by *B.abortus, B.suis* and *B.melitensis* may occur occasionally when dogs eat placentas from infected farm animals. The disease is most commonly transmitted sexually and bitches abort at 40 to 60 days of gestation (Foster and Smith, 2008).

2.8.1 Brucellosis in cattle, sheep and goats in Sudan:

B.melitensis was isolated from cow's milk in El-Gezira, central Sudan (Daffalla and Khan, 1958). The disease in Darfur states, Western Sudan, appears to be widely spread. Musa *et al.* (1990) reported the prevalence of the disease in different animal species including cattle and concluded that the highest prevalence was in intensive farming systems and under nomadic conditions. Cattle were found most affected (13.9%) followed by camels (7.76%), goats (5.98%) and sheep (3.5%). The prevalence was found to range between 14-26 % in South Darfur, which is known to be the richest state in animal population in the country. *Brucella* organisms isolated from South Darfur state were identified and typed as *Br.abortus* biovar 6 (Musa, 1995).

In West Darfur state the disease was studied only by Musa, (1995) in two provinces (Wadi Saleh &Zalingi). In Zalingi, goats were found to be most affected (16.9%) followed by sheep (13.2%) and cattle (8.8%). In Wadi Saleh, the disease was studied only in cattle (12.2%).

2.8.2 The disease in sheep and goats:

Sheep and goats brucellosis (excluding *Brucella ovis* infection which is not pathogenic for humans) is a zoonotic infection with important effects on both public and animal health and production and is widespread in many areas of

the world, particularly in some Mediterranean and Middle Eastern countries. *Brucella melitensis*, the main etiologic agent of brucellosis in small ruminants, was the first species in the genus *Brucella* described. It was first isolated by Bruce in 1887 (Alton, 1990) from the spleens of soldiers dying of Mediterranean fever on the island of Malta. Bruce called it *Micrococcus melitensis*. The origin of the disease remained a mystery for nearly 20 years until it was discovered that goats were the source of infection for humanpopulations. Brucellosis in sheep and goats is rarely caused by *Br.abortus* (Garin-Bastuji *et al*., 1994) or *Br.Suis* (Paolicchi *et al*., 1993).

2.9 Human brucellosis:

Human brucellosis is normally associated with the consumption of milk and other animal products contaminated with *Brucella* organisms from infected animals primarily ruminants such as cattle and goats (CDC, 2000a; 2000b). The people at risk are usually laboratory workers, veterinarians, farm, and slaughter house workers (Young, 1995). The symptoms are inconsistent fever, sweating, weakness, anaemia, headaches, depression and body pains (Roushan *et al*., 2006). The duration of the disease varies from a few weeks to months or even years (Sauret-Vilissova, 2002). These symptoms are similar to those associated with many other febrile diseases (Pappas

et al., 2003).). The duration of the disease varies from a few weeks to months or even years (Sauret-Vilissova, 2002).

2.10 Manifestation

2.10.1 Animal manifestation

In animals the disease is characterized by abortion, premature birth, dead or weak calves as well as loss in milk production (Alton, 1990).

After exposure to the bacteria, clinical manifestations may appear within 5 to 60 days. Most infected patients present with acute disease consisting of General symptoms, such as fever, malaise, sweats and lymphadenopathy and/or hepatosplenomegaly. However, a subset of patients developschronic brucellosis, a more severe form of the disease that can be associated with osteoarticular signs including spondylitis, arthritis and osteomyelitis, or genitourinary changes, such as orchitis, epididymitis, glomerulonephritis and kidney abscesses. Life-threatening complications comprise, in descending order of frequency, neurobrucellosis, liverabscesses, and endocarditis. (Xavier et al, 2010).

In humans, brucellosis often occurs through contact with infected animals

Or materials and through skin abrasions. Symptoms in human brucellosis

Can be highly variable, ranging from non-specific, flu-like symptoms (acute Form) to undulant fever, arthritis, orchitis, epididymitis, fatigue, malaise ,chills, sweats, headaches, myalgia, arthralgia, and weight loss. (Xavier et al, 2010).

2.10.2 Human manifestation

In humans, *B.melitensisis* the most pathogenic and invasive species followed by *B.suis* and *B.abortus* (Bricker and Halling, 1994). The symptoms take one to three weeks to manifest but sometimes can takeseveral months. Patients usually manifest septicaemia, prolonged undulating fever, chills, profuse sweating and high temperatures (Falagas and Bliziotis, 2006). In advanced cases, there is encephalitis, meningitis, peripheral neuritis, spondylitis, suppurative arthritis and vegetative endocarditis (Agarwal et al., 2000).Patient temperatures vary, ranging from normal (about 37ºC) in the morning to 40ºC inthe afternoon. The most common symptoms are general malaise, insomnia, arthralgia, headache, anorexia, constipation and sexual impotence. Depression, nervousness and irritation present evidence of a marked effect on the nervous system. The duration of the disease varies from weeks or months to several years. Theduration has been reduced to some extent by improved supportive treatment which hasalso resulted in a reduction in the incidence of relapses. Some of the treatment strategies

include a combination of injectable deoxymicin (12.5%), streptomycin and rifampicin for 6 months. Some Tuberculosis first line drugs can also be used to treat brucellosis and these are ethambutol, pyrazinamide, rifampicin and streptomycin (Mantur *et al*., 2007).

2.11 Diagnostic methods of Brucellosis:

The diagnosis of human brucellosis cannot be made solely on clinical grounds due to wide variety of clinical manifestation of this disease ,and it is essential to perform bacteriological and serological testes (Mantur and Amarnath ,2008). Apresumptive identification of *brucella* isolate at genus level can be made on the basis of colonial morphology ,appearance of smears stained with the methods of Gram and Stamp stains, and the result of oxidase and slide agglutination tests with *brucella* specific antisera. (Corbel, 2006). the definitive diagnosis of brucellosis is made when the organisms isolated fromblood, bone marrow, or other body fluids or tissues (Al-Eissa *et al*., 1999). Blood is best patient's material for successful *burcella* isolation .serum samples from acute disease phase are collected immediately, while other and etch next serum sample is collected after 14 to 21days (Zvizdic *et al*., 2006), RBPT is of value as a screening test especially in high risk rural areas where it is not possible to perform SAT (Mantuur and Amarnath, 2008). Among the newer serologictest, enzyme –linked immuonosorbal assay (ELISA) appears to be

the most sensitive and it may replace the SAT in future (Al-Eissa *et al*., 1999).

Molecular methods such as PCR-based assays are also available and are particularly useful in chronically infected patients where the yield of bacteria from blood cultures is usually low (Poester *et al*., 2010).

2.12 Control of Brucellosis:

There are a number of approaches in the brucellosis control and eradication programs which include vaccination of animals, surveillance, testing, quarantine and culling (Madkour, 2001). In some countries like the United Kingdom, the United States of America and Canada, animal vaccinations, surveillance, testing (serological and molecular based) and slaughter methods have essentially freed them from the disease for some years although there have been some incidental cases as a result of relaxation of the above mentioned control methods. The other factor is the increasing exchange of animals that may be harbouring *Brucella* organisms (Kiel and Khan, 1989). Animal vaccination in endemic areas has been the most effective control method. An attenuated vaccine strain that induce a T- cell mediated immune response grants a more improved immunity than killed vaccines (Tizard, 2000). In many countries, S19 vaccine was the only *Brucella* vaccine used for the control programmes until recently, RB-51 vaccine

has been introduced on the market which is also a live vaccine derived from rough strain of *B.abortus*. Standard brucellosis serological tests do not detect antibodies stimulated by RB-51 hence avoiding theproblem of detecting brucellosis-vaccinated animals testing positive (Ramirez *et al*., 2002). However, S19 is still the most effective vaccine used to control brucellosis. The attenuated strain is a live vaccine that ignites the immune response of the vaccinated animal to resist *Brucella* infection by producing antibodies against the attacking organisms and getting rid of the dead organisms by phagocytes. These antibodies produced against the disease disappear from the systemic circulation in a few months although lifelong immunity has been suggested so that the animal retains the resistance to disease for years (Tizard, 2000). In developing countries the S19 vaccine is still in use as it is easily produced. The disadvantage in its use has been the tendency to stimulate systemic clinical signs such as anorexia, drop in milk yield, oedema at the injection site, listlessness and high fever. Other signs may include abortion in pregnant cows, orchitis in bulls and febrile disease in humans (Tizard, 2000).

2.13 Treatment of Brucellosis:

Treatment is unlikely to be undertaken in animals. The use of long-acting oxytetracycline at 20 mg/kg body weight intramuscularly at 3-4 day intervals for 5 treatments in

combination with streptomycin at 25mg/kg body weight intramuscularly or intravenously daily for seven consecutive days was partially successful in the treatment of infected cows. The administration of oxytetracycline concurrently with vaccination may reduce the antibody response in cattle (Blood and Roddostitis, 1989). Radwann *et al.*(1987) pointed out that a long term treatment with a high dose of oxytetracycline (1000 mg/day per 6 weeks, I/P) had completely eliminated *Br.Melitensis* from naturally infected sheep. In humans however, many antimicrobial agents are used such as Tetracycline or Doxycycline, Trimethoprim, Sulfamethoxazole and Streptomycin (Young and Corbel, 1989).

2.14 Geographical distribution

B.meletinsis infection in sheep appears to occur endemically in theMediterranean region, especially along its northern and eastern shores stretching through Central Asia as far south as the Arabian Peninsula and asfar east as Mongolia. Parts of Latin America are also seriously affected, especially Mexico, Peru and northern Argentina. The disease also occurs in Africa and India. However, North America (except Mexico) is believed to be Free, as are Northern Europe (except for sporadic incursions from the south), Southeast Asia, Australia and New Zealand (FAO/OIE/WHO, 1997).

2.15 Published Studies on caprine brucellosis:

Brucellosis is an important bacterial zoonotic disease causing significant

Economic loss in dairy industries worldwide including Bangladesh. But limited studies are devoted to determine the prevalence of brucellosis in goat in all districts of Bangladesh. Therefore, a cross-sectional study was undertaken to determine the seroprevalence of brucellosis in Black Bengalgoats in NilphamariSadar and Kishoreganjupazillas of Nilphamari district of Bangladesh using Rose Bengal Test (RBT) as screening test and I-ELISA as confirmatory test. A total of 154 sera samples from Black Bengal goats were collected from Nilphamari district. Epidemiological data on the selected Black Bengal goats were collected using a structured questionnaire. The overall seroprevalence of brucellosis was found to be 2.59% in Black Bengal goats. A significantly ($p<0.01$) highprevalence of brucellosis was found in Black Bengal goats with the history of previous abortion (33.33%). An insignificant ($p>0.05$) but higher prevalence of brucellosis was found in adult Black Bengal goats (>24 months) than young. The prevalence was relatively higher in cross-bred than pure Black Bengal goats, in female than male and in pregnant than non-pregnant Black Bengal goats. The result of the study will provide baseline data for control of brucellosis in goat in Bangladesh (Rahman *et al*., 2012).

Also total of 242 milk and 208 blood samples of goat were collected from three organized goat farms and surrounding rural areas of Bangladesh Agricultural University to determine the prevalence and associated risk factors of brucellosis in Black Bengal goats during the period from December 2008 to September 2009. Milk samples were screened by Milk Ring Test (MRT) and serum samples by Rose Bengal test (RBT) and Micro Agglutination Test (MAT) for detection of *brucella* specific antibody in milk and blood respectively. The overall prevalence was recorded as 13.64% in milk by MRT; 3.85% and 3.37% in serum by RBT and MAT respectively. About 21.21(7/33) % and18.18 (6/33) % of MRT positive goat showed positive reactions in RBT and MAT respectively. Does aged up to 4 years had lower prevalence (3.70%) of brucellosis than those aged over 4 years (12.50%). About 2.1 (odds ratio, OR = 2.1; 95% CI: 1.21- 4.53) and 47.1(OR = 47.1; 95% CI: 5.3- 416.6) folds increased odds of seropositivity of brucellosis were observed in aborted and placental retention cases respectively. Significantly ($p<0.05$) higher prevalence of brucellosis was recorded at late lactation stage (17.94%) than those were in mid (16%) and early lactation stage (11.76%). A significantly higher odds of seropositivity of brucellosis was observed in does (OR = 23; 95% CI: 3.08-173.62). About 7 folds (OR = 6.8; 95% CI: 1.13- 5.32)

increased odds of seropositivity was observed in pregnant does. (Islam, *et al*., 2010).

Across - sectional study was conducted in Hammer and Dasenech Districts of South Omo Zone to determine seroprevalence of caprine brucellosis and its potential risk factors. Simple random sampling method was used to select 384 adult goats with no previous history of vaccination against brucellosis in the two districts. Modified Rose Bengal Plate Test (mRBPT) and complement fixation test (CFT) were used as screening and confirmatory tests, respectively. The results revealed that 16 goats (4.2%) were found seropositive for caprine brucellosis by mRBPT test and CFT. Seroprevalence of caprine (Tigist Ashagrie, *et al*., 2011).

Across sectional study was carried out on different Governorates representing all over Egypt to evaluate the potential major risk factors, mal- biosecurity practices and their role in the maintenance of the disease among farm animals. Serum samples (1670) were collected from 126 Herds / Flocks of sheep, goats and cattle and analyzed using Rose Bengal Plate test and iELISA test. A structured questionnaire was designed to identify and evaluate the role of risk factors for Brucellosis. The results pointed out that, prevalence of brucellosis among herds/flocks of sheep, goats and cattle were; 26.66%, 18.88% and 17.22%

respectively. And the seropositive percentages in blood samples were 21.20%, 14.5 % and 2.16% respectively. Major risk factors play a very important role in the prevention and maintenance of the disease among farm animals. The role and magnitude of risk factors varied but the presence of good sanitary measures in farms are considered as a protective factor, where R.R was less than 1 and the attributable risk was -0.01. [Nature and Science 2010; 8(5):190-197]. (ISSN: 1545-0740). (Kaoud *et al*., 2010).

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 Bangladesh. 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).

A cross-sectional study was conducted in the pastoral region of Afar, in eastern and central Ethiopia, to determine the distribution of brucellosis in small ruminants. Between December 2005 and June 2006, 1,568 serum samples were taken: 563 samples from sheep and 1,005 from goats. One hundred and forty-seven of these (9.4%) tested positive using the Rose Bengal plate test (RBPT), and 76 (4.8%) also tested positive by the complement fixation test (CFT). Brucellosis was detected in all five administrative zones of the region. The difference in prevalence (P) among the zones was not statistically significant (P > 0.05). The seroprevalence of *Brucella* infection was found to be 5.8% (n = 58) in goats and 3.2% (n = 18) in sheep. A prevalence rate of 5.3% was observed in adult animals and 1.6% in younger sheep and goats. Caprine species (_2 = 5.56) and adult goats and sheep (_2 = 4.84) were found to be at higher risk of *Brucella* infection (P < 0.05). No statistically significant difference was found between males and females (_2 = 2.57, P > 0.05). The study showed that

small-ruminant brucellosis is a widely distributed disease in Afar. The authors recommend the implementation of well-organized disease controland prevention methods to mitigate the economic losses and public health hazard caused by the disease. (Ashenafi, *et al*., 2007).

Chapter Three

Materials and Methods

3.1 Study area

This study was conducted in El-genaina witch is the Capital of west Darfour state. Western Darfur state is located in the western parts of the Sudan. It got borders with central Darfur state to the south east and north Darfur state to the north; it also shares international borders with Chad republic to the west. It has an area of 75,000 KM and a total human population of 757,000 engaged mostly in

agriculture and livestock rearing. The climate varies in western Darfur state, daily maximum temperature 38-40 ºC in May and the mean daily minimum temperature 12-16 ºCin February is about 25.2 C. There is a single rainy season, which occurs between June and October, but the peak of rainfall takes place between July and September. The rainfall ranges from low rainfall in the desert (180mm) in the northern part to the clay high rainfall; wood land (88mm) in the southern parts where the lowland is covered with broad leaves wooded savanna trees and grass. In summer (March - June) the climate is dry and hot, while in autumn (July - October) it is wet and warm. During winter (December-February), the climate is cool and dry.

3.2 Study design:

Across sectional study was conducted to estimate prevalence and risk Factors of Caprine brucellosis in El-genaina state during December 2014 -February 2015

3.3 Sampling Method:

The samples were collected by cluster sampling method from some farm and household.

3.4 Sample size determination and Sample collection:

3.4.1 Sample size determination:

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

Required sample size (n).

$$N = \frac{4*P*Q}{L2}$$

N= sample size

P= expected prevalence

L= desired absolute precision

Q= (1-P) (Martin *et al*., 1987)

The expected prevalence were considered as 6% in El-genaina state

(Yousef, 2010).

The sample size were calculated as follow:-

$$N = \frac{4*(,06)*(0.94)}{(0.0025)} = 90 \text{ animals}$$

The small sample size calculated (90) was multiplied by 3 to increase precession of the results (Thursfield, 2007)

3.4.2 Sample collection:

About 10 mL of blood was collected form the jugular vein of 270 goats of different ages and sex from herd and was selected randomly from farm and household .Using plain vacationer tubes and was transported in thermo flask with minimal possible shaking and the serum samples was separated by centrifugation for 5000 r/5minutsand stored at -20°C until testing at ministry of Animal Wealth laboratory in El-genaina State.

3.5 Laboratory procedures:

The serum samples were first screened using standardized buffered Rose Bengal stained antigen obtained from Soba Laboratory Using the technique described by Alton *et al*., (1975).

3.5.1 Rose Bengal Plate Test (RBPT):

3.5.1.1Test method:

This was carried out using standard Rose Bengal plate test antigen obtained from Central Veterinary Laboratory, Khartoum, Soba according to the method of Alton *et al*., (1975). Equal volumes (0.03mls) of antigen and test serum were mixed thoroughly on the ceramic plate of the test using a tooth pick, and the plate was rocked by rocking machine for four minutes.

Control Setup. The positive and negative controls were set up, and the results of the serology were compared. Any

degree of agglutination was considered positive while absence of agglutination was regarded as negative.

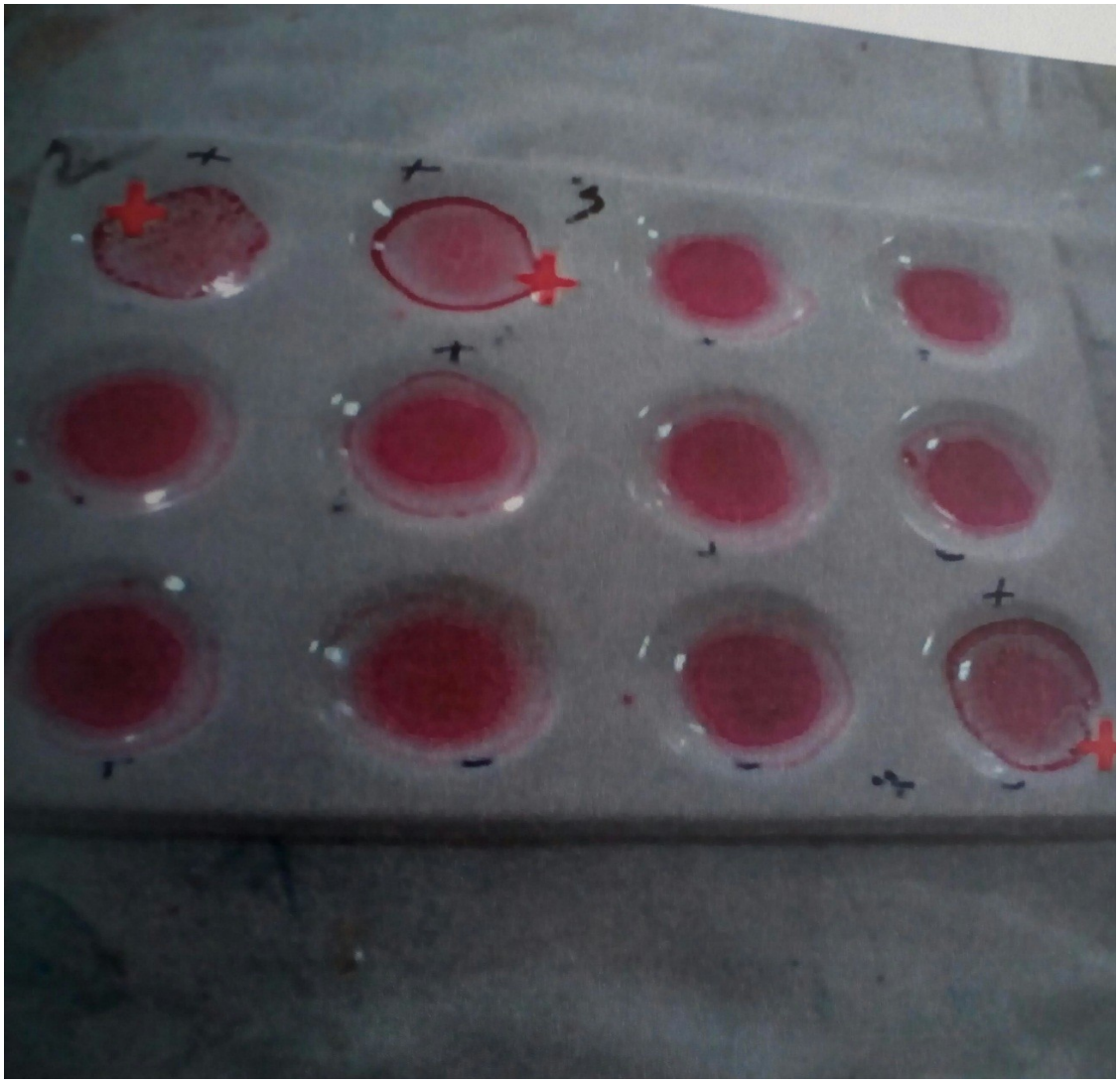

Figure 2: RBPT Result

3.6 Data collection:

The questionnaire was designed to elicit information about the factors associated with Caprine brucellosis. The risk

factors considered to be associated with goat brucellosis were selected after a review of the related published literature. The questionnaire had 10 questions; all questions were closed-ended type, and were classified to sex, age, breed, herd size, history of abortion, history of retained placenta, parity, type of production, sharing male, and contact with other animals.

3.7 Statistical analysis:

The data was collected from the field and the laboratory results was stored in the Microsoft excel spread sheet program, and the statistical analysis was performed using SPSS version 16.0 software program. The prevalence proportion was determined by considering the total number of animals tested and positive reactors by RBPT, using the formula given by Thrufield (2007). First the data was analyzed using descriptive statistics such as frequency cross tabulation table to determine the distribution of selected possible risk factors related to *brucella* infection. The associations between the outcome variable and potential risk factors was screened in a univariable analysis using Chi-square test. A risk factor with a P-value ≤ 0.25was considered significant and then entered to multivariable analysis using Forward Logistic Regression to determine the main factors associated with the occurrence of brucellosis. The odds ratio (OR) was used to measure the

strength of association between the factor and the outcome. Result was illustrated in tables showing Exp B, 95% confidence interval and *p-value* Variable with a *P-value* ≤ 0.05 was considered statistically significant.

Chapter Four

Results

4.1 Results:

Of the total 270 goat inspected, only 13 (4.8%) animals were positive, and the restwere negative for Brucellosis (Table 1.1).

Table 1: Distribution of *Brucella* infection among 270 goats examined in El-genaina State:

	Result	Frequency	Percent	Cumulative Percent
Valid -ve	Negative	257	94.8	100.0
	Positive	13	4.8	4.8
+ve	Total	270	99.6	

4.2 Age of animal:

Two hundred seventy goat of various ages were examined in this study. The results showed the distribution of 270 goat examined for goat brucellosis by age. 101 of goat were less than two year, and 116 of goat were from 2-4 year, and 53 goat were more than 4 year. Rate of Infection was high in animals which were 2-4 years (8.62%). Rate of Infection in age less than 2 year was (2.97%), and in more than 4 years was (0%). The chi square test showed significant association between infection and age of animal. P -value = .029. (Table 4).

4.3 Sex of animal:

The result of this study showed the distribution of 270 goat examined for brucellosis by sex .Total number of female examined was 251 animals, while the total number of male examined was 19.Rate of infection within females was

(5.17%) (Table 3). And rate of infection within male was (0%) (Table 3). The chi square test showed no significant association between infection and age of animal. P-value=.309. (Table 4).

4.4 Breed of animal:

The results of study showed distribution of brucellosis by breed. Total number of local goat examined was 255 among those 13 were infected. Rate of infection within local goat was (5.98%). And total number of cross was 15. Rate of infection within cross goat was (0%). (Table 3).The chi square test showed no significant association between infection and ageanimal. P-value=0.370. (Table 4).

4.5 History of abortion:

The result of this study showed the distribution of 270 goat examined for brucellosis by abortion.Total number of animal aborted was 25, among those 6 were found infected.Rate of infection within aborted animals was (24%).While the total number of animal not aborted was 245, among those 7 were found infected. Rate of infection within not aborted was (2.85%). (Table 3).

The chi square test showed highly significant association between infection and history of abortion in animals. P-value= .000 (Table 4).

4.6 History of retrained placenta:

The result of this study showed the distribution of 270 goat examined for brucellosis with retrained placenta. Total number of animal with no retrained placenta was 262 animal, while the total number of animal with retrained placenta examined was 8 animal. Among noretrained placenta 12 animal were found infected. Rate of infection was (4.58%). Among retrained placenta, 1 animal was found infected. Rate of infection was (12.5%). (Table 3).The chi square test showed no significant association between infection and Presenceof placenta in animals. (P-value = . 303). (Table 4).

4.7 Parity:

The result of this study showed the distribution of 270goat examined for brucellosis byparity. Total number of non-parity animals examined was 80 animals, while the total number of animals 1-3 was 131 animals. And number of animals more than 3times examined was 59 (table 3). Among non-parity, 6 animals were found infected. Rate of infection within non parity (7.5%) (Table 3). Among animals 1-3 times, 7 animals were found infected. Rate of infection within animal’s 1-3 times was (5.37%). Among animals more than 3 times, No animals wasfound infected.. Rate of infection within animals more than 3 times was (0%). (Table 3).

The chi square test showed significant association between infection and Parity of animal. (P-value= .115) (Table 4)

4.8 Type of production:

The result of this study showed the distribution of 270 goat examined for brucellosis by type of production. Total number of animal used for meat production was 64 animals, while the total number of animals used for milk production was 105 animal and total number of mixed production was 101 (table 3). Among meatproduction, 3 animals were found infected .Rate of infection within meat was (4.68%).Among milk production, 9 animals were found infected. Rate of infection within milk production was (8.57%). Among mixed production 1 animal was found infected. Rate of infection was (0.990%). The chi square test showed significant association between infections and Type of production. (P-value=.040 (Table 4).

4.9 Sharing male:

The result of this study showed the distribution of 270 goat examined for brucellosis by sharing male. Total number of animal with sharing male examined was 48 animals, while the total number of animals with no sharing male examined was 203 (Table 3). Among sharing male, 5animals were found infected. Rate of infection was (10.41%). Among no sharing male, 8 animals were found infected. Rate of infection was (3.60%).

(Table 3). The chi square test showed significant association between infections and sharing male (p-value = .046), (Table 4).

4.10 Contact with other animals:

The result of this study showed the distribution of 270 goat examined for brucellosis by Contact with other animals.Total number of animal with contact with other animals was 67 animals, while the total number of animals with no contact with other animals examined was 203 animals (Table 3)

Among contact with other animals, 4 animals were found infected. Rate of infection was (5.97%). Among nocontact with other animals, 9 animals were found infected. Rate of infection was (4.43%). (Table 3) The chi square test showed no significant association between infections and Contact with other animals (p-value = .610), (Table 4).

4.11 Herd size:

The result of this study showed the distribution of 270 goat examined for brucellosis by herd size. Total number of animal with less than 10 animals was 94, while the total number of animals with more than 10 animals examined was 176 animals. (Table 2). Among those of less than 10 animals, 4 animals were found infected. Rate of infection was (4.25%). Among animals with more than 10 animals, 9

animals were found infected. Rate of infection was (5.11%). (Table 3).

The chi square test showed no significant association between infections and Herd size. (P-value=.754) (Table 4).

Table 2: Summary of frequency tables for potential risk factors of Brucellosis among 270 goats examined at El-genaina:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %

Age			
<2	101	37.3	37.4
2-4	116	42.8	80.4
>4	53	19.6	100.0
Sex			
female	251	92.6	93.0
male	19	7.0	100.0
Breed			
Local	255	94.1	94.4
Cross	15	5.5	100.0
Abortion			
Yes	25	9.2	9.3
No	245	90.4	100.0
Placenta			
Yes	8	3.0	3.0
No	262	96.7	100.0
Parity			
Non	80	29.5	29.6
1-3	131	48.3	78.1
>3	59	21.8	100.0

Table 2 Continued:

Type of production			
meat	64	23.6	23.7
milk	105	38.7	62.6
mixed	101	37.3	100.0
Sharing male			
Yes	48	17.7	17.8
No	222	81.9	100.0
Contact with other animal			
yes	67	24.7	24.8
No	203	74.9	100.0
Herd size			
≤10	94	34.7	34.8
>10	176	64.9	100.0

Table 3: Summary of cross tabulation for potential risk factors of Brucellosis among 270 goats examined at El-genaina State:

Risk factors	No. inspected	No. affected (%)
Age		
<2	101	3 (2.97)
2-4	116	10 (8.62)
>4	53	0 (0)
Sex		
Female	251	13 (5.17)
male	19	0 (0)
Breed		
Local	255	13 (5.98)
Cross	15	0 (0)
Abortion		
Yes	25	6 (24)
No	245	7 (2.85)
Retained placenta		
Yes	8	1 (12.5)
No	262	12 (4.58)

Table 3: Continued:

Parity		
Non	80	6 (7.5)
1-3	131	7 (5.34)
>3	59	0 (0)
Type of production		
Meat	64	3 (4.68)
Milk	105	9 (8.57)
Mixed	101	1 (0.990)
Sharing male		
Yes	48	5 (10.41)
No	222	8 (3.60)
Contact with other animal		
Yes	67	4 (5.97)
No	203	9 (4.43)
Herd size		
≤10	94	4 (4.25)
>10	176	9 (5.11)

Table 4: Summary of univariate analysis for potential risk factors of goat brucellosis in El-genaina State-Darfour-Sudan:

Risk factors	No .inspected	No affected (%)	Df	X^2	p-value
Age			2	7.097	.029*
< 2years	101	3 (2.97)			
2-4years	116	10 (8.62)			
>4	53	0 (0)			
Sex			1	1.034	.309
Female	251	13 (5.17)			
Male	19	0 (0)			
Breed			1	.803	.370
Local	255	13 (5.17)			
Cross	15	0 (0)			
Abortion			1	22.127	.000*
Yes	25	6 (24)			
No	245	7 (2.85)			

History of retrained placenta Yes No	 8 262	 1 (12.5) 7 (2.85)	1	1.062	.303

Table 1.4 Continued:

Parity Non 1-3 >3	 80 131 59	 6 (75) 7 (5.34) 0 (0)	2	4.323	.115*
Type of production Meat Milk Mixed	 64 105 101	 3 (4.86) 9 (8.57) 1 (0.990)	2	6.459	.040*
Sharing male Yes No	 48 222	 5 (10.41) 8 (3.60)	1	3.997	0.046*

Contact withother animal Yes No	 67 203	 4 (5.97) 9 (4.43)	1	.260	.610
Herd size **≤ 1**0 >10	 94 176	 4 (4.25) 9 (5.11)	1	.098	.754

*means significant value .p- value ≤0.25

Table 5: Multivariate analysis of potential risk factor of goat brucellosis in Elgenaina State-Sudan:

Risk factor	**No.tested**	**Positive %**	**OR**	**Cl 95%**	**P-value**
Age(years)					.064
	101	3 (2.97)	.134	.025-.720	
< 2	116	10 (8.62)	8.16	.000 - 0	
2 – 4	53	0 (0)	8	Ref	
> 4			Ref		
Abortion					.000*
Yes	25	6 (24)	Ref	Ref	
No	245	7 (2.85)	35.011	6.188-198.085	

Parity					.152
Non	80	6 (75)	4.99	.984-25.323	
1-3	131	7 (5.34)	1	.000-0	
>3	59	0 (0)	1.22	Ref	
			7		
			Ref		
Type of productio n					.344
	64	3 (4.86)	.538	.095-3.059	
Meat	105	9 (8.57)	2.59	.192-35.125	
Milk	101	1 (0.99)	7	Ref	
Mixed			Ref		
Sharing male					.067
Yes	48	5 (10.41)	Ref	Ref	
No	222	8 (3.60)	4.02	.905	
			8	-17.930	

* means significant value .p- value ≤0.05

Five risk factors were analyzed by stepwise forward Logistic Regresstion using brucellosis –seropositivity as an outcome .the multivariable Logistic Regresstion model identified abortion (p-value=0.000) as risk factors significantly associated (p-value≤0.05)with *brucella* seropositivity.

Chapter five
Discussion

Result of obtained from the present study showed that an overall prevalence of rate of antibodies against brucellosis in goat serum samples collected from four directions (north, south, east, west) in El-genaina State were found to be 4.8% by RBPT.The result of RBPT is in

agreement with the findings ofthe prevalence rate observed in this study using RBPT is higher than those rates reported by Omer *et al*.,(2007) which was 2.1%, and El.Ansary, (1999) where was 4% in Kassala area, eastern Sudan .Rayas, (2004) which was 0.3% in Nyala area southern Darfur state, sudan ,Ahmed ,(2004) which was 0.45% in Red sea State, Sudan, Ashargie *et al*.,(2011) which was 4.2%. and 1.9% recent report by Bekele *et al*., (2011) from Jijiga, Eastern Ethiopia. However, The result of the present study is lower than that in Afar Region of Teshale, (2006) who reported prevalence of 16.55%in Ethiopia, Ahmed, (2010) who reported prevalence of 31% Libya, Al-Majali, (2005) who reported prevalence of 27.7% in Jordan, Hawari, (2011) who reported prevalence of 24.6% Jordan. 9.8 % in goats at public livestock farm in Pakistan (Arshad *et al*., 2011), Bertu,(2010) who reported prevalence of 16.1% in Nigeria, and Negash, (2011) who reported close prevalence of 11.3% in Egypt.These differences could bemainly due to variation in agro-ecological location, management and production systems, differences in sampling methods and serogical test employed.

Few studies in the Sudan have addressed risk factors with Sero-positivity to brucellosis in goat. In current study, univariate analysisusing Chi square, with confidence interval of 95% at a P-value of ≤0.25 was used to identify potential risk factors associated with RBPT-positivity for

brucellosis infection in goat. Significant risk factors associated with RBPT positive in the Univarite analysis were found to be Age, (p= 0.029), Abortion (p= 0.000) Parity (P=0.115), Sharing male (p=0.046) and Type of production (0.040)

The present study revealed that there was statistically significant difference among Age, this finding was higher in 2-4 years (8.62) than <2 years and >4.This result is agreement with Negash *et al*., (2011), who has been reported that The susceptibility to brucellosis appear to be more commonly associated with sexual maturity. Sexually mature and pregnant animals are more prone to *brucella* infection (Radostits *et al*., 2006).

The study revealed that there was no significant difference between males and females' goat, which prevalence of brucellosis was higher in female (5.17) than in male. This finding supports the observation of Chandra *et al*. (2005) and Rahman *et al*. (2011). But in contrast with that reported by Islam *et al*., (2010).

The higher prevalence in female could be attributed to the fact that female sex hormones and erythritol stimulate the grow and multiplication of *Brucella.* (Radostits *et al*., 2006). Furthermore, male animals areknown to be less susceptible to *Brucella infection* due to less amount of carbon 4-sugar erythritol (Hirsh and Zee, 1999)

The breed wise distribution of brucellosis was shown in (Table3). An insignificantly higher prevalence was found in local breed than cross goat (0%).

The statistically significant association between seroprevalence of caprine brucellosis and occurrence rate of abortion and parity number could be explained by the fact that abortions and prolonged kidding interval (parity) are typical out-puts of brucellosis (Radostits *et al*., 2000). This result agree with (Ashagrie *et al*., 2011).

No significant difference was observed in relationship between brucellosis and history of retained placenta .This result disagrees with Islam *et al* (2010), and Ashagria *et al* (2011). Although a higher prevalence was found in goat with retained placenta (24%) than goat with not retained placenta(2.85%).This could be explained by the fact that the infection localizes in the placenta and lead to the development of placentitis, with subsequent abortion, and after abortion uterine infection persists for up to 5 months, (Radostits*et al*.,2006).

A significant association was observed in goats for milk production rather than meat production or both meat and milk production.This result is in agreement with Coelho *et al.*,(2008) who reported significant association with milk

production rather than meat production .but this result disagrees with Coelho *et al*.,(2004).

No significant difference was observed in contact with other animals. The study revealed higher prevalence rate in animals which have contact with other animals species in the same place compared to those kept in separate place.similar finding were reported by Al-Majali.*et al*., (2005).

A significant difference was observed in goat sharing males. This could be explained by the fact that infected male my discharge semen containing *brucella* organisms and it is likely to transmit the infection to the does. Similar finding was recorded by Lithg-pereira *et al*., (2004).
No significant difference was observed in herd size. This result was disagrees with Coelho *et al*., (2004). However the herd more than 10 goats showed higher prevalence than herd with less than 10 goats. This result could be attributed to the fact that large herds tend to be raised under intensive management system, which may increase the possibility of transmission of the disease through direct contact.

Conclusion:

The study provides information to the veterinary outhorities to launch more epidemiological studies including isolation and identification of *brucella* organism which cause the disease, the knowledge that is useful for control of the disease .The important risk factor for the infection with brucellosis in this study was abortion cases.

Our result highlight the need for further research, including more risk factors that possibly related to the brucellosis prevalence.

Recommendations:

- Isolation of *Brucella* from goat in the State is important for epidemiological and control policies.
- Vaccination programs should be attempted to control the disease.
- 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.

- 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.
- Numbers of samples used in this study were too small compared to the animal populations sampled, so, it's recommended that, Samples sizes should be increased in further researches.

References

1. Agarwal, N., A. Joshi, N. Jain, B. V. Sharma and D.K. Kochar. (2000). Human Brucellosis: A Grossly Under

diagnosed Disease in India (A prospective, study of 75 cases). Department of Medicine, SP Medical College, Bikaner.

2. Ahamed, M.M.H.(2004). Studies on animal's brucellosis in Red sea State, Sudan, Master Thesis, Faculty of Veterinary Medicine , University of Khartoum, Sudan.

3. Ahmed, O.M., Elmeshri, E.S,Abuzweda, R.A., Blau,M., Abouzeed, M.Y.,Ibrahim, A., Salem,H., Alzwam,F., Abid.,Elfahem,A., and Elrais ,A(2010). Seroprevalence of bruceiiosis in animals and humans population in the Western mountains region in Libya, Euro Surveill, Vol.15(30):PP.19625.

4. Akbarmehr, J., and Ghiyamirad, M. (2011). Serological survey of brucellosis in livestock animals in Sarab City (East Azarbayjan province), Iran. African Journal of Microbiology Research, Vol. 5(10), pp. 1220-1223.

5. Al-Eissa, A. Y. (1999).Brucellosis in Sadui Aribia: past, present, and future Annals of Saudi Medicine, Vol.19, pp, 403-405.

6. Al-Majali AM.2005. Seroepidemiology of caprine brucellosis in Jordan. Small Rumin Res 58, 13-18.

7. Alton, G. G., (1990). *Brucella melitensis*. In: "Animal brucellosis" (Nielsen, K., J.R. Duncan eds).CRC Press. Boston, Page 383-40

8. Alton, G.G., Jones, I.M. and Pietz, D.E. (1975). Laboratory Techniques In Brucellosis. World Health Organization. Monograph SeriesNo.55, second edition.

9. Alton, G. G., L. M. Jone, R. D. Angus and J. M. Verger. (1988). Bacteriological methods, in laboratory techniques in brucellosis, 2nd Ed. World Health Organisation, Geneva, Page 11-64.

10. Amato Garcia, A. J. (1995). The return of Brucellosis. Maltese Journal, 7: 7 –8.

11. Anonymous (1997). WHO meeting on development of new/improved animal brucellosis Vaccines. Mediterranean Zoonosis Control Centre, Information Circular No. 44 December.

12. Anonymous (2002). Bovine Brucellosis Chapter 2.3.1. Office International Des Epizootics: Paris, France.

13. Arshad M, Munir M, Khan HJI, Abbas RZ, Rasool MH, Rahman KU and Khalil N (2011). Seroprevalence of brucellosis in goats from public and private livestock farms in Pakistan. *Journal of Veterinary Research.*

14. Ashagrie, T., Deneke, Y., and Tolosa T. (2011). Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. African Journal of Microbiology Research, Vol.5 (13), PP. 1682-1476.

15. Ashenafi F, Teshale S, Ejeta G, Fikru R and Laikemariam Y (2007). Distribution of brucellosis among

small ruminants in the pastoral region of Afar, eastern Ethiopia. *Revue Scientifique et Technique.* 26: 731-739.

16. Bandara, A. B. and M. B. Mahipale. (2002). Incidence of brucellosis in Sri Lanka: An overview. Veterinary Microbiology, 90: 197-207.

17. Bekele M, Mohammed H, Tefera M, Tolosa T (2011). Small ruminant Brucellosis and community perception in Jijiga District, Somali
Regional State, Eastern Ethiopia. Trop. Anim. Health. Prod., 43: 893.

18. Benkirane A.2006. Ovine and caprine brucellosis: World distribution and control/ eradication strategies in West Asia/North Africa region. *Small Rumin Res* 62, 19-25.

19. Bertu, J.W., Ajogi, I., Bale, O.O.J., Kwaga, P.K.J., and Ocholi, A.R.. (2010). Seroepidemology of brucellosis in small ruminants in plateau State, Nigeria. Afar J.Microbiol .Res, Vol.4 (19), PP 1935-1938.

20. Blood, D.C. and Radostits, O.M. (1989).*Veterinary Medicine*. 7th ed. Bailliere Tindall, London, pp. 677-696.

21. Bricker, B. J and B. M. Halling. (1994). Differention of *Brucella abortus* by 1, 2, and 4 *Brucela melitensis, Brucella ovis* and *Brucella suis* by 1 by PCR. Journal of Clinical Microbiology, 32: 2660.

22.Cadmus, S. I. B., I. F. Ijagbone, H. E. Oputa, H. K. Adesokan and J. A. Stack, 2006. Serological survey of

brucellosis in livestock animals and workers in Ibadan, Nigeria. African J. Biomed. Res., 9: 163 – 168

23. Cassataro J, K Pasquevich, L Bruno, JC Wallach, CA Fossati, PC Baldi. **2004**. Antibody reactivity to Omp31 from *Brucella melitensis* in human and animal infections by smooth and rough *Brucellae*. *Clin.Diagn Lab Immunol*11, 111-114.

24. Centers for Disease Control and Prevention. (2000a). Suspected brucellosis case prompts investigation of possible bioterrorism – related activity: New Hampshire and Massachusetts, 1999. MMWR: Morbidity and Mortality Weekly Report, 49: 509 – 512.

25. Centers for Disease Control and Prevention. (2000b). Biological and Chemical terrorism: strategic plan preparedness and response. MMWR: Morbidity and Mortality Weekly Report, 49: 1 – 4.

26. Chandra M, Singh BR, Shankar H, Agarwal M, Sharma G, Agrawal RK and Babu N (2005). Seroprevalences of brucellosis in chevon goats from Bareily slaughter house*. Indian Journal of Animal Science* 75: 220-221.

27. Cloeckaert, A., J. M. Verger, M. Grayen, J. Y. Paquet, B. Garin-Bastufi, G. Foster and J. Godfroid. (2001). Classification of *Brucella spp* isolated from marine mammals

28. Cloeckaert, A., J. Y. Vizcaino Nipaguet, R. A. Bowden and P. H. Elzer. (2002). Major Outer membrane proteins of *Brucella* spp therefore past, present and future. Veterinary Microbiology, 90: 229-247.

29. Coelho, M.A., Coelho, C.A., Gois,J., Pinto, D.M., and Rodrigues, J. (2008). Multifactorial correspondence analysis of risk factors for sheep and goats brucellosis Seroprevalence, Small ruminant Research, Vol.78,pp181-185.

30. Coelho, M.A., Coelho, C.A., Roboredo, M., and Rodrigues, J.(2004).A case –control study of risk factors for brucellosis seropositivity in portuguese small ruminants herds. Preventive Veterinarny Medicine, Vol. 82, pp 291-301.

31. Coghlan, J. D. (1995). *Brucella*. In: Greenwood D., Slack R.C.B, Peuther J.F. (eds) Medical Microbiology. A microbiology guide to Microbial infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. Page 325 – 328.

32. Corbel, M. J. (1997). Brucellosis an overview. Emerging Infections, 3: 213-221.

33. Corbel, M.J. (2006): Brucellosis in humans and animals. Produced by the World Health Organization in collaboration with the Food and

Agriculture Organization of the United Nations and World Organization for Animal. Health WHO/CDS/EPR/2006.7.

34. Dafalla, E.N. and Khan, A.Q. (1958). The occurrence, epidemiology and control of animal brucellosis in the Sudan. *Bull Epizoot. Dis. Aft.,* **6**: 243-247.

35. Diaz–Aparicho, E., C. Marin, B. Alonso–Urmeneta and V. Arogon. (1994). Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. Journal of Clinical Microbiology, 32:1159 – 1165.

36. El-Ansary ,H,E., Mohammed, A.B., Hamad, A.A., and Karom, O.A.(1999). Brucellosis among animals and human contacts in Eastern Sudan , Saudi Medical Journal, Vol.(7),pp577-579.

37. Enright, F. M. (1990). The pathogenesis and pathology of *Brucella* infections in Domestic animals. Research in Veterinary Science, 60: 48-50.

38. Erdenebaatar J, B Bayarsaikhan, AYondondorj, M Watarai, T Shirahata, E Jargalsaikhan, K Kawamoto, S Makino. 2004.

Epidemiological and serological survey of brucellosis in Mongolia by ELISA using sarcosine extracts. *Microbiol Immunol* 48, 571-577.

39. Falagas, M. E. and I. A. Bliziotis. (2006). Quinolones for Treatment of Human Brucellosis: Critical Review of the Evidence from Microbiological and Clinical Studies. Antimicrobial Agents and Chemotherapy, 50: 22-33.

40. FAO/OIE/WHO (1997) 1995 Animal Health Yearbook, FAO Animal Production and Health Series, FAO, Rome, Italy.

41. Finlay, B. B and S. Falkow. (1989). Common themes in Microbial Pathogenicity. Microbiology Reveiws, 53: 210 – 230.

42. Foster, R and M. Smith. (2008). Brucellosis (*Brucellacanis*) and Abortion in Dogs. Biology Work group-Citizedium.

43. Garin-Bastuji, B., Gerbier, G., Douzal, Y., Vaucel, D., Hummel, N., Thiébaud, M., Grayon, M. and Verger, J.M. (1994). La brucellose animale en France en 1993. *Epidemiol. Sante Anim.,* **26**: 103-130.

44. Godfroid, J. (2002). Brucellosis in wildlife. Review of Science Technological Office of International Epizootics, 21: 277-286.

45. Godfroid, J., C. Salgerman and V. Wellemansa. (2002). How to substantiate eradication of bovine brucellosis when a specific serological reaction occurs in the course of brucellosis testing. Veterinary Microbiology, 90:461-477.

46. Hawari, D.A.(2011). Epidemiological studies, prevalence and risk factors of brucellosis in sheep and goats in South province of west Bank, Asian Journal of animals and veterinary advances,Vol.7(6),pp535-539.

47. Hirsh DC, Zee YC (1999). Veterinary Microbiology. Blackwell Science,UK, pp. 196-203.

48. Islam, A.M., Samad, A.M., and Rahman, A.M.K.A. (2010). Risk factors associated with prevalence of brucellosis in black Bengal goats in Bngladesh, Bangl. J. Vet. Med., Vol. 8(2) pp 141 – 147.

49. Kaoud, A.H., Zaki, M.M., El-Dahshan, R.A., and Nasrm, A.S. (2010). Epidemiology of Brucellosis among Farm Animals, Nature and Science, Vol. 8 (5), pp 190-197.

50. Kiel, F. W and M. Y. Khan. (1989). Brucellosis in Saudi Arabia. Social Science Medicine, 29: 999 – 1001.

51. Latimer, E. J., N. Simmers, R. M. Srirangamatthan, I. T. Roop, G. G. Schuring and S. M Boyle. (1992). *Brucella abortus* difficient in Copper/Zinc superoxide dismutase isvirulent in BALB/c mice. Microbiology and Pathogenesis, 12:105 – 113.

52. Lithy-pereira, L.P., Rojo- Vazquez, A.F., and Mainar-Jaime, C.R.(2004) case -Control study of risk factors for high within –flock small-ruminant brucellosis prevalence in a brucellosis low-prevalence area, Epidemiol .infect, Vol.132,pp 201-210.

53. Madkour, M. M. (2001). Madkour's brucellosis. Barlin: Berlin and Heiderberg: springer – Verlay 306.

54. Mantur, B. G., S. K. Amarnath and R. S. Slinde. (2007). Review of clinical and laboratory features of human brucellosis. Indian Journal of Medical Microbiology, 25: 188-202.

55. Mantur G.B., and Amrnath, K.S.(2008). Brucellosis in India-a review, J.Biosci. Vol 33 (4), pp. 539-547.

56. Martin, w., Meek, H.A., and Willeberg, p. (1987): Veterinary
Epidemiology principles And Methods, Second printing, United
State of America.

57. McDermott, M., B. O'Connell, T. E. Mulvinill and E. C. Sweeing. (1994). Chronic *Brucella* infection of the supra-patellae bursa with sinus formation. Journal of Clinical Pathology, 47: 764 – 766.

58. Megersa,B., Biffa, Abunna,F., Regassa,A., Godfroid,J., and Skjerve,E. (2008). Seroprevalence of brucellosis and its contribution to abortion in cattle, camels, and goats kept under pastoral management in Borana, Ethiopia, trop Anim Health Prod, Vol,43, pp 651-656.

59. Musa, M.T. (1995). Brucellosis in Darfur State, The Magnitude of the problem and methods of diagnosis and control. *Ph.D. Thesis,* University of Khartoum.

60. Musa, M.T.; Jahans, K.L. and Fadalla, M.E. (1990). Brucellosis biovars isolated from Nomadic cattle in the Southern Darfur province in Western Sudan. *J. Comp. Path.,***102**: 46-54.

61. Negash,E., Shimelis,S., and Beyene,D. (2011). Seroprevalence of small ruminant brucellosis and it is

public health awareness in selected sites of Dire Dawa region Eastern Ethiopea ,Journal of Veterinary Medicine and Animal Health ,Vol.4(4),pp61-66.

62. Neto FG, Y Vaz.2002. Conjuntival Rev. 1 vaccination of adult sheep and goats in Trás-os-Montes, Portugal. *Epidémiolet Santé Anim*42, 99-107.

63. OIE Terrestrial Manual (2009): Chapter 2.4.3. — Bovine brucellosis.

World Organization for Animal Health (OIE).

64. O.I.E. (1996).*Manual of Standards for Diagnostic tests and Vaccines*. 3rd ed., Office International of Epizooties 1997. Paris, France. Caprine and ovine brucellosis, pp. 350-362; Bovine brucellosis, pp. 242-25

65. Omer,M.M., Abdelaziz,A.A., Abusalab, A.M.S., and Ahmed, M.A (2007). Survey of brucellosis among sheep, goats, camels, and cattle in Kassala area, Eastern Sudan, Journal of Animal and Veterinary Adavances, Vol.6 (5), pp 635-637.

66. Paolicchi, F.A., Terzolo, H.R. and Campero, C.M. (1993). Isolation of *Brucellasuis* from the semen of a ram. *Vet. Rec.,* **132**: 67.

67. Pappas, G., M. Bosilkovski, N. Akritidis, M. Mastora, H. Ketena and E. Tsianos. (2003). Brucellosis and the Respiratory system. Clinical Infectious Diseases, 37: 95-99.

68. Parker, R. (2007). Diseases affecting Reproduction in Beef Cattle. College of Agriculture and Home Economics New Mexico State University. USA.

69. Poester, P.F., Nielsen, K., Samartino, EL., and Yu, L. W (2010). Diagnosis of brucellosis, the Open Veterinary Science Journal, Vol .4, pp: 46-60.

70. Purcel, K.B.L., Hoover, L.D. and Friedlander, M. A (1997). Brucellosis,*Medical Aspects of Biological Warfare* (Online). pp: 185-198.

71. Quinn, P. J., M. E. Carter, B. Markey and G. R. Carter. (1999). Clinical Veterinary Microbiology. Edinburgh: Mosby International limited, Page 261 – 267.

72. Radostits, M.O., Gay,C.C., Hinchcliff,W.K., and Constable

,D.P. (2006).Veterinary medicine : a textbook of the disease of cattle, sheep, pigs, goats and horses,10th Edition, London, Baillier and Tindal , pp. 991-993.

73. Radostits OM, Gray CC, Blood DC and Hinchliff KW (2000). Brucellosis. In: Veterinary Medicine, Text Book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9th ed., Baillere Tindal, London, pp. 867-891.

74. Radwan, A.I.; Hafez, S.M.; Al Aska, A.K.; Al Xamani, M.J.; Bekairi, S.I.; Julaifi, M.A. and Al Mukayel, A.A. (1987). Experimental treatment of bovine brucellosis with oxytetracycline alone or combined with

streptomycin. *XXIII World Veterinary Congress*, Montreal Quebec, August 16-21, 1987. p.14.

75. Rahman, S.M., Faruk. O.M., M., Kim, Y.J., Kang,I,S., and Jung,C.S (2011): Prevalence of Brucellosis in ruminants in Bangladish ,Veterinarni Medicina, Vol.56 (8), pp379-385.

76. Rahman, S.M., Her, M., Kim, Y.J., Kang, I.S., Lee, K., Uddin, J.M., Chakrabartty, A., and Jung, C.S. (2012). Brucellosis among ruminants in some districts of Bangladesh using four conventional serological assays, African Journal of Microbiology Research Vol. 6 (22), pp. 4775-4781.

77. Ramirez, M., M. E. Hamdy and M. Amin. (2002). Serologic response and time to eradication in herds with brucellosis vaccinated with strain 19 or strain RB-51. Arch.Med.Vet, 34: 143-151.

78. Rayas,A.R. (2004). Studies on caprine brucellosis in Nyala area, South Darfour, State, Master Thesis, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

79. Rege, J. E. O., A. M. Nyamu and D. Sendalo (eds). (2006). The role of Biotechnology in animal agriculture to address poverty in Africa; opportunities and challenges. Proceedings of the 4th All Africa conference on Animal Agriculture and the 31st Annual meeting of Tanzania Society for Animal Production Arusha, Tanzania.

80. Riley, D. K and D. C. Robertson. (1984). Ingestion and intercellular survival of *Brucella abortus*in human and bovine polymorphonuclear leucocytes. Infectious Immunology, 46: 224 – 230.

81. Roushan, M. R. H., M. Mohraz, M. Hajiahmadi, A. Ramzani and A. A. Valayati. (2006). Efficacy of gentamycin plus doxycycline versus streptomycin plus doxycycline inthe treatment of brucellosis in Humans. Clinical Infectious Diseases, 42: 1075-80.

82. Rust, R. S. (2006). Brucellosis. In: Shah A.K., F. Talavera, D, Parma, F.P, Thomas, S.R. Benbadis and N. Lorenzo, (Eds). E Medicin especialisties, website: http//medscape.com.

83. Sauret, J. M and N. Vilissova. (2002). Human Brucellosis. Journal of American Board of Family Practice, 15: 401-405.

84. Stack, J. A and A. P. Macmillan. (2000). Identification and Biotyping of *Brucella* spp. Clinical and Vaccine Immunology, 146:166-167.

85. Stuart, F. A., M. Corbel and R. A.Brewer. (1987). Experimental *Brucella abortus* infections. Veterinary Microbiology, 14: 365-379.

86. Teshale, S., Muhie , Y., Dagne, A ., and Kidanemariam, A.(2006): Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali patoral areas practice, Revue Med.Vet., Vol.157,pp 557-563.

87. Thrusfield, M. (1995): Veterinary Epidemiology, Second Edition by
Black Well Science Ltd.

88. Thrusfield, M. (2007). Veterinary Epidemiology, 3rd edition, Oxford: Black Well Science.

89. TigistAshagrie, YosefeDeneke and TadeleTolosa (2011)
Seroprevalence of caprine brucellosis and associatedrisk factors in South Omo Zone of Southern Ethiopia. African Journal of Microbiology Research Vol. 5(13) pp. 1682-1476.

90. Tizard, I. R., (2000).Veterinary immunology: An introduction. 6th Edition, Philadephia, Pennsylviania 19106: W.B Saunders Company. Page 238-265.

91. Traboulsi, R., I. Uttman and S. S. Kani. (2007). Prepatellar *Brucella melitensis* bursits: Case report and literature review. Clinical Rheumatology, 11:1941-1942.

92. Villafane–Lastra, T., R. Bergogao and J. F. Garcia. (1948). 1st Inter-American Congress on Brucellosis, 441-63 (Ediciones Del Hospital General. Secretarial desalubridady. Asistencia, Mexical).

93. Xavier, N.M., Paixão, A.T., den Hartigh, B.A., Tsolis, M.R. and
Santos, L.R. (2010): Pathogenesis of *Brucella* spp. *The Open*
*Veterinary Science Journal, **4**:* 109-118.

94. Young,E.H. (1995). An Overview of human brucellosis. Clinical Infectious Disease, 21: 283-290.

95. Young,E.J. and Corbel,M.J. (1989). *Brucellosis Clinical and Laboratory Aspect*. CRC press, Florida, USA, pp.163-172.

96. Zvizdic,S, Cengic,D, Bratic,M, Mehanic,S., Pinjo,F ., and Hamzic,S (2006). *Brucella melitensis*, review of human infection case, Bosnian Journal of basic Medical Sciences ,Vol .6 (1) ,PP.15-18.

Appendices

Appendix I

Questionnaire Format for caprine Brucellosis survey in Elgenina state, Darfur, Sudan:-

Date…

Herd owner……..

Owner information: .1-sex: male ( ) female ( )

Animal identification:

1-Sex: Male ( ) Female ( )

2-Age: <_ 2 years ( ) 2 -4 years ( ) > 4 years ( )

3-Breed: local ( ) cross ( )

4-History of abortion: Yes ( ) No ( )

5-History of retrained placenta: Yes ( ) No ( )

6-parity: Non ( ) 1-3 ( ) > 3 ( )

7- Type of production : Meat ( ) Milk ( ) Mixed ( )

8-Sharing male: Yes ( ) No ( )

9- Contact with other animal: Yes ( ) No ( )

10-Herd size: <_ 10 ( ) > 10 ( )

Appendix 2

Frequency tables for potential risk factors of Brucellosisamong 270 goat examined at El-genaina:

Age:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Age			
<2	101	37.3	37.4
2-4	116	42.8	80.4
>4	53	19.6	100.0

b. Sex:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Female	251	92.6	93.0
male	19	7.0	100.0

c. Breed

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Local	255	94.1	94.4
Cross	15	5.5	100.0

d. Abortion

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Yes	25	9.2	9.3
No	245	90.4	100.0

e. presence of retained Placenta:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Yes	8	3.0	3.0
No	262	96.7	100.0

f. Parity:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %

Non	80	29.5	29.6
1-3	131	48.3	78.1
>3	59	21.8	100.0

g. Type of production

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Meat	64	23.6	23.7
Milk	105	38.7	62.6
Mixed	101	37.3	100.0

h. Sharing male

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Yes	48	17.7	17.8
No	222	81.9	100.0

i. Contact with other animal:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %

Yes	67	24.7	24.8
No	203	74.9	100.0

j. Herd size:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
≤10	94	34.7	34.8
>10	176	64.9	100.0

Appendix 3

Summary of cross tabulation for potential risk factors of Brucellosis among 270 goat examined at Elgeniana:

Age:

Result	Age			Total
	<2	1-4	>4	
+ve	3	10	0	13
%	3.0%	8.6%	.0%	4.8%
-ve	98	106	53	257
%	97.0%	91.4%	99.0%	95.2%
Total	64	105	101	270
%	100.0%	100.0%	100.0%	100.0%

Sex

	Sex		Total
Results	Female	Male	
+ve	13	0	13
% of sex	5.2%	0%	4.8%
- ve	238	19	257
% of sex	94.8%	100.0%	95.2%
	251	19	270
Total	100.0%	100.0%	100.0%

Breed

	Breed		Total
Results	Local	Cross	
+ve	1	0	13
%	5.1%	0%	4.8%
- ve	242	15	257
	94.9%	100.0%	95.2%
	255	15	270
Total	100.0%	100.0%	100.0%

Abortion

	Abortion		Total
Results	Yes	No	
+ve	6	7	13

%	24.0%	2.9%	%
- ve	19 76.0%	238 97.1%	257 95.2%
Total	25 100.0%	245 100.0%	270 100.0%

History of retrained placenta:

Result	History of retrained placenta		Total
	Yes	No	
+ve	1	12	13
%	12.5%	4.6%	4.8%
-ve	7	250	257
%	87.5%	95.4%	95.2%
Total	8	262	270
%	100.0%	100.0%	100.0%

Parity:

Parity

Result	Non	1-3	>3	Total
+ve	6	7	0	13
%	7.5%	5.3%	.0%	4.8%
S-ve	61	96	100	257
%	95%	91.4%	99.0%	95.2%
Total	64	105	101	270
%	100.0%	100.0%	100.0%	100.0%

Type of production:

Type of production

Result	Meat	Milk	Mixed	Total
+ve	3	9	1	13
%	4.7%	8.6%	1.0%	4.8%
-ve	61	96	100	257
%	95%	91.4%	99.0%	95.2%
Total	64	105	101	270
%	100.0%	100.0%	100.0%	100.0%

Sharing male:

Result	Sharing male	Total	
	Yes	No	
+ve	5	8	13
%	10.4%	3.6%	4.8%
-ve	43	214	257
%	89.6%	96.4%	95.2%
Total	48	222	270
%	100.0%	100.0%	100.0%

Contact with other animals:

Result	Contact with other animals	Total	
	Yes	No	
+ve	4	9	13
%	6.0	4.4%	4.8%
-ve	63	194	270
%	94.0%	95.6 %	95.2%

Total %	67 100.0%	203 100.0%	270 100.0%

Herd size:

Result	**Herd size**		Total
	≤10	>10	
+ve %	4 4.3	9 5.1%	13 4.8%
-ve %	90 95.7%	167 94.9%	270 95.2%

Appendix 4

Association between caprine brucellosis infection and potential risk factors using the Chi- square test:

Chi-Square Tests for age

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.097[a]	2	.029
Likelihood Ratio	9.093	2	.011
Linear-by-Linear Association	.071	1	.790
N of Valid Cases	270		

Chi-Square Tests for sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.034[a]	1	.309
Likelihood Ratio	1.946	1	.163
Linear-by-Linear Association	1.030	1	.310
N of Valid Cases	270		

Chi-Square Tests of breed

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.803[a]	1	.370
Likelihood Ratio	1.524	1	.217
Linear-by-Linear Association	.800	1	.371
N of Valid Cases	270		

Chi-Square Tests of abortion

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	22.127	1	.000
Likelihood Ratio	13.107	1	.000
Linear-by-Linear Association	22.045	1	.000
N of Valid Cases	270		

Chi-Square Tests of presence of retained placenta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.062[a]	1	.303
Likelihood Ratio	.761	1	.383
Linear-by-Linear Association	1.059	1	.304
N of Valid Cases	270		

Chi-Square Tests of parity

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.323[a]	2	.115
Likelihood Ratio	6.983	2	.030
Linear-by-Linear Association	3.939	1	.047
N of Valid Cases	270		

Chi-Square Tests of type of production

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.459[a]	2	.040
Likelihood Ratio	7.368	2	.025
Linear-by-Linear Association	1.944	1	.163
N of Valid Cases	270		

Chi-Square Tests of sharing male:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.997[a]	1	.046
Likelihood Ratio	3.276	1	.070
Linear-by-Linear Association	3.982	1	.046
N of Valid Cases	270		

Chi-Square Tests of contact with other animals:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.260[a]	1	.610
Likelihood Ratio	.248	1	.619
Linear-by-Linear Association	.259	1	.611
N of Valid Cases	270		

Chi-Square Tests of herd size:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.098	1	.754
Likelihood Ratio	.100	1	.751
Linear-by-Linear Association	.098	1	.754
N of Valid Cases	270		