DEDICATION

To:

My mother;

My father;

My brothers and sisters;

My sons and daughters

Soul of My husband

All friends and colleagues;

I dedicate this work with love and affection.

Acknowledgement

First of all, my thanks and praise are due to almightily Allah.

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Abstract:-

The Seroprevalence ,risk factors and economic impact of bovine brucellosis were determined in cattle in Khartoum State Sudan . Total 1286 sera were prepared after collecting blood samples from the animals in Localities, Khartoum (n=144) ,Omdurman (n=238), Karry (n= 109) , Umbada (n=208) Bahry (n=312), and (n=275) Shargelnel Localities . Out of 1286 serum samples tested , 332 samples were positive to Rose Bengal Plate Test. All sera positive to RBPT (332 samples) were subjected to further confirmatory test using competitive Enzyme Linked Immunosorbent Assay . (c-Elisa) .The prevalence of bovine brucellosis in Khartoum State was recorded as 25.8% . In localities the prevalence were 33.3% in Khartoum, 30.8% in Umbada ,29.5% in Bahary , 23.1% in Omdurman, 22.9% Shergelneel and 9.2 % in Karry locality .

The questionnaire was designed to collect information on individual herds from the animal owners. A total of 14 risk factors such as age, sex, herd size ,geography, history of abortion , history of vaccination, mixed farming, type breed, mixed age, calves bar, breeding methods (natural ,artificial) ,presences of veterinary services , awareness, and water supply were investigated. The results of the univariate Chi-square analysis revealed that seropositivity to brucellosis was significantly higher in 11 risk factors (locality, herd type, breed, veterinary services, vaccination, awareness, bull share ,water source ,housing , age , and gender $P < 0.25$). were found to be associated with bovine brucellosis seroprevalence. Herd size, abortion History , breeding method were not identified as the risk factors associated with seropositivity to Brucellosis. No one of these risk factors

were significant (P >0.25), Attributed the endemic status of brucellosis in Khartoum State.

From the known methods of economic impact assessment of animal disease partial budgeting according to Morris (1999) was considered to be the best method to evaluate the economic impact of an endemic disease. From the result estimation of the financial loss due to brucellosis in the whole State, the highest economic losses in Bahary locality was 14,240.343 SDG, Umbada locality was 13.787.557 SDG ,Shergalneel locality was 10.766.726 SDG ,Omdurman locality was 5.293.608 SDG , Khartoum locality was 2,484.239 SDG and Karry locality was 1.378.688 SDG

The mass losses was milk due to brucella effect on milk production .The highest economic losses of milk in Bahary locality 9,150.455 SDG, Umbada locality was 7,319,060 SDG, Shergalneel locality was 6,129,830 SDG, Omdurman locality was 5954692 SDG , Khartoum locality was 5,123,442and Karry locality was 0,765,295 SDG.

Calves losses due to abortion was calculated in the State localities Khartoum, Omdurman, Karry, Umbada , Bahary, and Shergalneel .There were 32,000 SDG , 48.000SDG ,08,000SDG , 64.000SDG , 32,000SDG, 16.000 SDG respectively .

The result indicated that the economic loss dut to infertility by brucella (repeat breeding) minor losses in localites Khartoum, Omdurman, Karry, Umbada , Bahary, and Shergalneel 3.726 , 4.960 , 0,000, 6.200, 8.680, 6.200 SDG respectively .

The estimation of the financial loss due to brucellosis in the whole State was 95,5964347SDG = 20.781226 U$$.

The results of this study showed that brucellosis is widely distributed and an endemic disease in Khartoum State.

ملخص البحث

الانتشار المصلي ,عوامل الخطر والأثر الاقتصادي لمرض البروسيلا في الابقار حددت في قطعان الماشيه بولاية الخرطوم .

اجمالي 1286 مصل تم الحصول عليها بعد جمع عينات الدم من محليات ولاية الخرطوم (الخرطوم 4 11, ام درمان 238, كرري 109 ,امبده208 , بحرى 312 وشرق النيل 5 27عينه) .

من العينات اعلاه (1286) عينة مصل كانت نسبة الاصابة 233 (25.8%) اجابيه لداء البروسيلا باسيخدام لوحة الروزبنغال. جميع العينات الاجابيه لروزبنغال خضعت فحص تاكيدي اليسا C لتاكيد اجابيتها . كانت نسبة الاصابه لداء البروسيلا بمحليات ولاية الخرطوم .محلية الخرطرم 33%, محلية امبده 30.8% .محلية بحري 29.5% ,محلية امدرمان 23.1% محلية شرق النيل 22.9% ومحلية كرري 09.2%.

تم جمع معلومات عن الحيوانات فردي و14من عوامل الخطر علي مستوي المزرعة وخصائص الانتاجيه الاخري باستخدام استبيان. كشفت النتائج تحليل الكاي المتغير ان ايجابية المصل لداء البروسيلا كانت عاليا وذات دلاله في 11 من عوامل الخطر هي المحليه, نوع القطيع , الهجين , الخدمات البيطريه, التطعيم ,وعي المنتجين , المشاركه في الثيران ,مصادر المياه ,المساكن ,العمر ,و الجنس كل هذه العوامل ساعدت في اجابية المصل لداء البر وسيلا في الابقار ($p<05$)

اما العوامل حجم القطيع تاريخ الاجهاض ونوعية الهجين ليست لها دلاله في اجابية المصل لداء البروسيلا في الابقار ($p>05$) يعزو الي ان مرض البروسيلا مستوطن بولاية الخرطوم.

لتقييم الاثر الاقتصادي لمرض البروسيلا اختيرت طريقة Morris (1999) وهي من افضل الطرق لتقييم الاثر الاقتصادي للامراض المستوطنه .

من النتائج تقيم الفاقد المالي بسبب داء البروسيلا بولاية الخرطوم , اعلي فاقد اقتصادي في محلية بحري بلغ 14,240,343 جنيه سوداني ومحلية امبده 13,785,576 جنيه سوداني و

محلية شرق النيل 10,766,764 جنيه سوداني ومحلية امدرمان 5,293,608 جنيه سوداني ومحلية الخرطوم 2,484,239 جنيه سوداني ثم محلية كررى 1,378,688 جنبة سوداني.

كتلة الفاقد في الحليب وذلك لتاثير البروسلا في انتاج الحليب اعلي نسبه فاقد مالي للحليب في محلية بحري 9,150450 جنيه سوداني تليها محلية ام بده 7,319066 جنيه سوداني تليها محلية شرق النيل 6,129830 جنيه سوداني تليها محلية ام درمان 5,954692 جنيه سوداني تليها محلية الخرطوم جنيه سوداني 5,123441 ومحلية كرري 0,765,295 جنيه سوداني .

تم حساب الفاقد في العجول بسبب الاحهاض في محليات الولايه, محلية الخرطوم ام درمان , كرري ,ام بده , بحري وشرق النيل 32000 ,8000,48000 ,64000, ,16000,32000 جنيه سوداني .

النتائج اظهرت ان الفاقد الاقتصادي لعدم الخصوبه بسبب مرض البروسيلا(اعادة التلقيح) فاقد ثانوي في المحليات الخرطوم ,ام درمان ,كرري , ام بده , بحري وشرق النيل 3726 ,4960 , 0000 ,6200, 8680 , 6200 جنيه سوداني .

التقيم للفاقد المالي الناشي عن مرض البروسيلا في ولاية الخرطوم 95,936,434 جنيه سوداني تعادل 6 20,78122 دولار امريكي

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Introduction:

Brucellosis is one of the world's major zoonotic problems. Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand it remains an uncontrolled problem in regions of high endemicity such as Africa, Mediterranean countries, Middle East, parts of Asia and Latin America (Refai , 2002).

Several synonyms of brucellosis have been known like Malta fever, undulant fever, Rock of Gibraltar fever and Bang's disease. The disease has a very old history, as organisms resembling *Brucella* had been detected in carbonized cheese from the Roman era. Brucellosis was first recognized as a disease affecting humans on the Island of Malta in the early 20th century. Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health programme (Capasso, 2002).

Brucellosis is caused by members of the genus *Brucella*. These are small, non-motile, aerobic facultative intracellular, Gram-negative coccobacilli.The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity . The species of *Brucella* and their major hosts are *Br. abortus* (cattle), *Br. melitensis*(goats, camels) *Br. suis* (swine) and *Br. ovis* (sheep). *Br.abortus* also causes infection in horses and is commonly found in chronic bursal enlargements as secondary invader rather than a primary pathogen (DeMassis *et al* 2005 OIE 2008).

The susceptibility of animals to brucellosis depends on their natural resistance, age, level of immunity, and environmental stress (Capasso,

2002).. Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection. Considering the damage done by the infection in animals in terms of decreased milk production ,abortions, weak offspring, weight loss, infertility and lameness, it is one of the most serious diseases of livestock. It is also a major impediment for the trade. Death may occur as a result of acute metritis, followed by retained fetal membranes (Radostits *et al.*, 2007).

Brucellosis in cattle is characterized primarily by abortion late in pregnancy, frequently followed by fetal membrane retantion and endometritis which may be the cause of infertility in subsequent pregnancies (Ahmad *et al*. 2009).

Transmission of brucellosis occurs mainly by ingestion of contaminated feed and water by the organisms which are present in large numbers in aborted fetuses, fetal membranes, and uterine discharges. However, infection through injured/intact skin, the mucosa of the respiratory system, and conjunctiva occurs frequently (Kebede *et al*. 2008). The *Brucella* may enter the body through digestive tract, lungs or mucosal layers and intact skin. Then it may spread through blood and the lymphatic system to any other organ where it infects the tissues and causes localized infection (Lapaque *et al.*, 2005). Bulls that are themselves infected and discharge semen containing organisms are mostly unlikely to transmit the disease, but the chance spread from the bull is very great if the semen is used for artificial insemination (Eshetu *et al*. 2005).

From public health view point, brucellosis is considered to be an occupational disease that mainly affects slaughter-house workers, butchers,

and veterinarians, infection of *Br. abortus, Br. meltinsis and Br. suis* in man is generally acute and may be followed by spontaneous recovery (Lamontagne *et al.,* 2010) . After a variable incubation period ranging from less than one week to several months, non-specific systemic symptoms such as fever, headache, malaise, night sweating and arthralgia follow, resembling a flu like disease. During the early stage of the disease, patients are frequently bacteraemic that has a continuous pattern, making circulating *Brucella* easily detectable by blood culture. Once in the blood stream, the organism is seeded to multiple organs/systems, especially those rich in reticuloendothelial tissue, such as liver, spleen, skeletal and hematopoietic system (Greenfield *et al.*,2002).

There are so many factors that can affect the prevalence of brucellosis in various species of livestock. Prevalence of brucellosis can vary according to climatic conditions, geography, species, sex, age and diagnostic tests applied. The seroprevalence of bovine brucellosis in east Africa as summarized by Asfaw *et al.* (1998) shows that in Djibouti, it was 4%; Somalia, 11.9%; Kenya, 19%; Rwanda, 34.9%; Sudan, 6.5–22.5%; and Uganda, 1.8%.

Brucellosis in Sudan was first reported from human cases as early as 1908 (Haseeb,1950) while ,*Br. abortus* was first isolated from dairy farm in Khartoum (Bennett, 1943). Many surveys were later conducted in different part of the Sudan and the results, showed that bovine brucellosis exists in almost all over of the Sudan.

Animal health care is very important in Khartoum state because of the high demand for animal products particularly milk, urban nature of the population, presence of foreign residents from other countries particularly

employers in international and regional organization, The income level is higher than other state leading to conductive environment for dairy products consumption, hence the progressive growth of dairy industry Ministry (2011).

The present study was therefore, carried out to determine the prevalence of bovine brucellosis and to know the risk factors and economic impact of bovine brucellosis in Khartoum state localities.

General Research objectives:

The study was planned to investigate and to draw attention to the status of Brucellosis in Khartoum State.

Special Research objectives:

- To estimate the prevalence rate 0f bovine brucellosis among dairy cattle in Khartoum State localities.
- To identify the risk factors associated with the occurrences bovine brucellosis.
- To investigate the economic impact due to bovine brucellosis.

Chapter One

Literature Review

1-1. Brucellosis:

1-1-1 . History and nomenclature

Under the name Malta fever, the disease now called brucellosis first came to the attention of British medical officers in the 1850s in Malta during the Crimean War. The causal relationship between organism and disease was first established in 1887 by Dr. David Bruce .In 1897, Danish veterinarian Bernhard Bang isolated *Brucella abortus* as the cousae agent; and the additional name Bang's disease was assigned. . Wilkinson, (1993).

Maltese doctor and archaeologist Sir Themistocles Zammit earned a knighthood for identifying unpasteurized milk as the major source of the pathogen in 1905, and it has since became known as Malta Fever. In cattle, this disease is also known as contagious abortion fever and infectious abortion. The popular name undulant fever originates from the characteristic undulance (or "wave-like" nature) of the fever, which rises and falls over weeks in untreated patients. In the 20th century, this name, along with brucellosis (after *Brucella*, named for Dr. Bruce), gradually replaced the 19th century names Mediterranean fever and Malta fever. The following obsolete names have previously been applied to brucellosis: Brucelliasis , Bruce's septicemia ,Chumble fever, continued fever, Crimean fever, Cyprus fever, febris melitensis, febris undulans, Fist of mercy, goat fever, melitensis septicemia, melitococcosis, milk sickness, mountain fever,

Neapolitan fever, Satan's fever, slow fever, Scottish Delight, Jones Disease.(Wilkinson, 1993).

In 1989, neurologists in Saudi Arabia discovered neurobrucellosis, eurological involvement in brucellosis.(Malhotra, 2004).

1-1-2:- Taxonomy and taxonomical controversies :-

The genus *Brucella* belongs to the family *Brucellaceae* within the order *Rhizobiales* of the class *Alphaproteobacteria* . The closest phylogenetic neighbor of the genus *Brucella* is the genus *Ochrobactrum*, a saprophyt that occasionally infects humans .Until 1985,the genus *Brucella* encompasses of 6 species, *B. melitensis, B. abortus, B. suis, Brucella ovis, Brucella neotomae* and *B. ovis*, known as the six classical species .All these *Brucella* species are genetically highly related. Verger et al.(1985) the proposed combination of the six species into a single species, *B. melitensis,* with the other species to be recognized as biovars (e.g., *B. melitensis* biovar abortus1) In 2003, the Subcommittee on the Taxonomy of *Brucella* unanimously agreed on are turn to the pre-1986 taxonomic treatment of the genus *Brucella*, implying reapproval of the six classical *Brucella* nomenspecies with their corresponding biovars .Osterman et al,(2006). Since2007, *Brucella ceti and Brucella pinnipedialis* (infecting preferentially cetaceans and pinnipeds, respectively) are recognized as new *Brucella* species Foster et al.,*(*2007). In2008, another new *Brucella* species, i.e., *Brucella microti* was first isolated in the common vole Scholz et al.,(2008b) and lastly, *Brucella inopinata* was recently isolated from a breast implant infection in an elderly woman with clinical signs of brucellosis Scholz et al.,(2010). This species is the only one that has not

been isolated from any animal reservoir.To date the genus *Brucella* encompessees ten recognized species. Prospective *Brucella* species have also been isolated from three native rat species in Australia, but not yet been included in the genus as well as in association with two cases of stillbirth in non-human primates Tiller *et al*.,(2010).

1-1-3. Species identification and biotyping

Brucella species are highly monomorphic, with minimal genetic variation among species and maintain a close taxonomic relationship and can only be distinguished by rigorous metabolic, immunologic, and biochemical analyses The similarities among the *Brucella* species extend to the genetic level at which all species share greater than 90 % DNA homology (Tiller *et al*., 2010) .Species of *Brucella* were differentiated in the laboratory by colonial morphology, growth requirement, various biochemical tests and lysis by bacteriophage .The accurate distinction between *Brucella* species and their biovars is performed by differential tests based on phenotypic characterization of lipopolysaccharide antigen, phage typing, dye sensitivity, CO2 requirement, H2S production and metabolic properties (Alton *et al.,* 1988).

1-1-3-1. BRUCELLA SPECIES

1-1-3-1-1. Br . MELITENSIS

CO2 independent. Produces no H2S, or no more than a trace, on peptone media. Usually grows in the presence of basic fuchsin and thionin. Usually hydrolyses urea. Oxidizes L-alanine, L-asparagine, L-glutamic acid, D-glucose, and i-DL-citrulline, DL-ornithine or L-lysine. Usually pathogenic for sheep and goats but may infect cattle and man. *Br. Melitensis* biovar

1,2.3 are well established and used for epidemiological purposes .The refrance strain biovar 1.

1-1-3-1-2. *BRUCELLA ABORTUS*

Usually requires supplementary (5%) CO2 for growth, especially on primary

isolation. Usually hydrolyses urea and produces moderate amounts of H2S but some strains may not. Usually grows in the presence of basic fuchsin, some biotypes will also grow in the presence of thionin and some are inhibited by both dyes. Oxidizes L-alanine, L-asparagine, L-glutamic acid, L-arabinose, Dgalactose, D-glucose, D-ribose and i-erythritol. Does not oxidize D-xylose, Larginine, DL-citrulline, DL-ornithine or L-lysine.

Usually pathogenic for cattle, causing abortion; can also infect other species including sheep, goats, camels, yaks, buffaloes, horses, dogs and man.

The recognize biotypes are (1,2,3,4,5,6,7and 9).

B. abortus biotype 8 is no longer recognized.(FAO/WHO) reference type strain is Br. abortion biovar1.

1-1-3-1-3. *BRUCELLA SUIS*

C 0 2 independent. Hydrolyses urea rapidly. Produces large amounts of H2S

or none at all depending upon biotype. Grows in the presence of thionin and usually inhibited by basic fuchsin but some strains grow on both dyes.

Oxidize D-ribose, D-glucose, i-erythritol, D-xylose, L-arginine, DLcitrulline and DL-ornithine. Do not usually oxidize L-alanine or L-asparagine. Oxidation of L-lysine, L-glutamic acid, L-arabinose and D-galactose varies with biotype. Usually pathogenic for pigs except for biotype 4 which is usually pathogenic for reindeer. May also infect other species including hares, rodents, dogs and man.

(FAO/WHO neotype and biotype reference strains :)

1-1-3-1-4. *BRUCELLA NEOTOMAE*

CO2 independent. Produces H2S. Hydrolyses urea rapidly. Does not grow in

the presence of basic fuchsin but will grow in the presence of thionin

(1:150,000). Smooth strains have the A surface antigen reactive in tests with

monospecific antisera. May produce acid from D-glucose, D-galactose, L-arabinose, and D-xylose in peptone water sugar. Oxidize L-asparagine, L-glutamic acid, Larabinose, D-galactose, D-glucose, i-erythritol and D-xylose. Do not oxidize L-alanine, L-arg¡nine, DL-c¡trulline, DL-orn¡th¡ne or L-lys¡ne. Oxidation of tuelalase variable. recaused in the desert wood rat *(Neotoma lepida* Thomas). Natural infections unknown in other species. No biotypes are recognized.

(FAO/WHO reference type strain :)

1-1-3-1-5. *BRUCELLA OVIS*

Requires supplementary (5-10%) C 0 2 for growth. H2S is not produced. Usually does not hydrolyse urea, but some strains may show weak activity after seven days. Grows in the presence of basic fuchsin and thionin. Does not reduce nitrate. A smooth phase does not occur, cultures are always in the

rough phase on primary isolation. Does not react with A and M monospecific ntisera but is agglutinated by R antiserum. Cross-reacts with *B. canis* and other non-smooth brucellae. Not lysed by phages Tb, Fi, Wb or Bk2 at any concentration. Lysed by phage R/C at RTD. Oxidizes L-alanine, L-asparagine and L-glutamic acid. Does not oxidize L-arabinose, D-galactose, D-glucose, D-ribose, i-erythritol, D-xylose, L-arginine, DL-

citrulline, DL-ornithine or Llysine. Adonitol is oxidized and this is useful for identification, as *B. ovis* and *B. neotomae* are the only species consistently active on this substrate. Pathogenicfor sheep causing epididymitis in ram and abortion in ewes. Natural infections are unknown in other species.

No biotypes are recognized. (FAO/WHO reference type strain) :

1-1-3-1-6. *BRUCELLA CAN/S*

CO2 independent. Hydrolyses urea rapidly. Does not produce H2S. Usually reduces nitrates but some strains may not. Usually grows on thionin but not on basic fuchsin. Cultures are always in the rough or mucoid phase on primary isolation. Does not react with monospecific antisera for A and M antigens but reacts with antiserum to R antigen. Cross-reacts serologically with *B. ovis* and other non-smooth brucellae. Not lysed by phages Tb, Fi, W b orBk2 at any concentration. Lysed by phage R/C at RTD. Oxidizes D-ribose, Dglucose, L-arginine, DL-citrulline, DL-ornithine and L-lysine. Does not oxidize L-alanine, L-asparagine, L-glutamic acid, L-arabinose, D-galactose or Dxylose. Oxidation of /-erythritol is variable.

Pathogenic for dogs causing epididymo-orchitis in the male and abortion and metritis in the female. May be transmitted to man. No biotypes are recognized.(FAO/WHO reference type strain):

1-1-4. Definition of the Brucellosis .

Brucellosis is an economically important disease in production animals worldwide Corbel, (1997*). Brucella melitensis, Brucella abortus* and *Brucella suis* cause abortion and infertility in their natural hosts, goats and sheep, cattle and swine, respectively. Albeit the irrespective host preferences, *Brucella* spp. Have also been isolated from a great variety of wildlife species. As a consequence, different wildlife species may act merely as spill-over hosts (victims) or as reservoir hosts (vectors) of *Brucella* spp. For other animal species and humans .Indeed, brucellosis is azoonosis and humans can acquire a debilitating febrile illness known as 'Mediterranean or undulant fever' ,as the result of contact with infected animals or consumption of their products Pappas *et al.,(*2006b).

1-1-5. *Brucella* life style—surviving immune system of the host

Within mammalian hosts, *Brucella* spp. Have an intracellular life style and infect both professional and non-professional phagocytes. The Vir Boper on, a type IV secretion pathway that Is induced on phagosomal acidification, plays a key role in intracellular parasitism and is essential for pathogenicity *Brucella* spp. will resist different environmental stresses in these phagocytic cells, modify their intracellular trafficking and eventually reach their replicative niche Kohler et al.(2002). *Brucella* spp. Survive and multiple in dendritic cells, interfere with their maturation, impair the antigen processing and thus compromises host immune responses (Roop *et al.*,2009). *Brucella* spp. Prevent apoptosis within the macrophage and their long term survival in the reticuloendothelial system of spleen, liver ,and bone marrow will sustain chronic infection (Gorvel and Moreno, 2002). During gestation, *Brucella* spp. Replicate in large numbers in placental

trophoblasts. The integrity of the placenta may be disrupted and abortion induced. The pregnant uterus is an immunological privileged site, which prevents the rejection of the fetus by modulating local immune responses which in turn may allow *Brucella* spp. to replicate extensively .The *Brucella* lipopolysaccharide (LPS) is a weak inducer of the host inflammatory cytokines compared to LPS molecules from many other Gram-negative bacterial pathogens Neta *et al.*, (2010).

1-1-6 Diagnosis of brucellosis

The diagnosis of brucellosis is confirmed by isolation and identification of the brucella organism. However, this approach is time-consuming, and the specific tests needed to characterize the bacteria are complicated. In order to be able to screen a large number of animals, the diagnostic tests should be 'inexpensive, easy to perform, rapid, highly sensitive and fairly specific'. Several serological tests have been designed to meet these requirements Godfroid , et al. (2010). recently produced a comprehensive review of the serological tests for brucellosis that are in common use ere written Therefore, within this section the most commonly used serological tests are only briefly summarized. Tests that are comparable (similar specificity and sensitivity as well as similar other characteristics) are grouped together These tests are:

a) Acidified antigen agglutination tests such as the rose- bengal /card test (RBT) and the buffered antigen plate agglutination test. These serological tests are simple to perform, inexpensive and suitable for screening individual animals Godfroid , et al. (2010).

b) Standard agglutination tests (SAT) such as the standard tube agglutination test and the seroagglutination test of Wright constitute another

group of tests that are comparable with each other. In the rest of this paper they are referred as the SAT-tests. According to Godfroid , et al. (2010). SAT tests are susceptible to producing false positive reactions.

c) The Complement fixation test (CFT) is another, separate test. The CFT is recommended by the OIE as the test prescribed for international trade CFT is often used as a second test for confirmation of RBT-positive sera Nielsen, et al(2007). .

d) Indirect enzyme immunoassays (ELISA) are the fourth serological test group that is often used to determine the prevalence of brucellosis in surveys. Recently developed ELISA tests are, according to highly sensitive, simple to use but expensive.(highlighted that the indirect ELISA is more sensitive than RBT tests and have a sensitivity of 100% and a specificity of 84.5%. Godfroid , et al. (2010). .

e) Milk ring test (MRT) is an adaptation of the agglutination test. This test is used to show if antibodies are present in the milk. . Mangen et al (2002).

1-1-7-. Treatment:-

In general, in adequate treatment is responsible for severe and debilitating chronic courses and long-term sequelae. Hence ,the basic therapeutic goal is not only to control acute illness but also to prevent complications and relapses .Use of at least two synergistic antibiotics, including doxycycline, rifampin ,streptomycin (or other amino glycosides) ortrimethoprim-sulfamethoxazole (cotrimoxazole), is therefore a must in prolonged chemotherapeutic regimens .Internationally two different treatment options are recommended. The combination of oral doxycycline 100 mg twice a day and rifampin600–900mg/day (15 mg/kg/day) in a single oral dose over

a 6-week course shows fewer adverse effects than a combination including streptomycin1 g intramuscularly once a day for 2 weeks or an alternative amino glycoside ,instead of the administration of revamping. However, the latter treatment is characterized by lower relapse rates Ariza etal.,(2007; Corbel, (2006). For a successful therapy of focal complications and chronic courses tripleortetra combinations of the antimicrobial drugs mentioned and longer treatment courses (>45days) are essential. Few cases of brucellosis in humans caused by *B. canis* have been described (Lucero *et al.*,(2010). However, canine brucellosis in man might be under diagnosed due to a low perception of the disease and a lack of valid serological tests. Human infections by marine mammal strains have a severe course but are reported only rarely. The clinical importance of *B. inopinat a* and the a typical *Brucella* strain (BO2) closely related to *B. inopinata* is still unclear despite the fact that both agents have been isolated from diseased human s(Scholz etal., 2010). Little is also known about the human pathogenicity of *B. microti* but in experimental cellular and marine models of infection *B. microti* exhibited a significantly higher virulence than other *Brucella* species (de Bagues *et al.*,2010).

1-1-8. Control:-

In the developed world, control of animal brucellosis has been successfully achieved through the combination of vaccination and test and slaughter programs (McDermott 2002; Pappas *et al.*,2006b), coupled with effective disease surveillance and animal movement control. In developing countries, however, control by test-and-slaughter is hardly achievable because of limited resources to indemnify farmers whose animals are slaughtered during such screening programs Since animals are not often

kept as business enterprises, the off takes are often low. Thus animals tend to live longer resulting in emotional attachment of the farmers to their animals. It has been suggested that any disease control strategies need to take into account the need and perceptions of the communities (Marcotty *et al.*,2009). While occupational exposure may be considered as a major mode of transmission, consumption of infected milk products from infected animals remains a major route of transmission even in non-endemic countries like Germany where consumption of *Brucella* contaminated products accounted for infections acquired abroad (Al Dahouk *et al.*,2005c). In a study done in Kampala, urban residents who had no contact with livestock were at risk of being *Brucella* infected an exposure attributed to consumption of raw milk products purchased from rural and peri-urban areaPasteurization or boiling of milk and milk products ,is likely to reduce human infections (Makita *et al.*,2008). .A survey conducted in Kenya showed that boiling of milk reduced the risk of exposure to *Brucella* . Other factors contributing to exposure included ignorance of risk of *Brucella* infection Marcotty et al.,*(*2009). In some cases, perceived enhanced nutritional qualities, taste, and health benefits have all been advocated as reasons for increased interest in raw milk consumption. Therefore, involvement of anthropologists and social workers will become increasingly important in successful control of human brucellosis Marcotty et al.,(2009). Risk assessment is a tool that should be advocated to the World Trade Organization in the context of trade policy (Agreement on the application of sanitary and phytosanitary measures).The methodology might also be used to assist in the choice of an appropriate national response strategy following an incursion of are emerging disease. The choice of a strategy in the affected regions should

be made after an independent, scientific and collective assessment where the range and magnitude of consequences of implementing or not, measures or surveillance programs of all susceptible domestic livestock (and possibly wildlife) are considered. Marcotty et al.,(2009). Generally, in most developed countries, test and slaughter programs, together with compensation for farmers, accreditation and financial incentives for disease-free herds, allowed the achievement of a status close to eradication of brucellosis in livestock and, consequently, in humans Saegerman et al., (2010). In cattle, the infection is predominantly caused by *B. abortus*, and is usually detected in pregnant females through abortions. The removal of sanitary barriers and the liberalization of exchanges in accordance with the World Trade Organization. agreement require the harmonization of the brucellosis health status among countries in order to eliminate the risk of contamination of a country with a favorable health status through importation of live animals and animal products from a country with a lower health status. In 2008, 12 EU member states were Officially Brucellosis Free(OBF) in cattle, as well as in sheep and goats. In 2008, 15 non-OBF member states reported bovine cases of brucellosis (herd prevalence equal to 0.12%).The situation is less favorable in Southern European countries but is still below 1%(European Food Safety Agency,2010b). Because brucellosis has public health and international trade implications, all member states have an interest in obtaining and in maintaining this officially free status. Cases of cross-infections with *B. melitensis* were observed in herds mixed with sheep and goat flocks in southern Europeand are regularly reported in the middle east where it has become an emerging veterinary and public health problem (Samaha *e t al.*, 2008).

1-1-9.vaccines:-

At the beginning of the 21stcentury Successful eradication programs have always been costly, long ,and hard to carry through. The difficulties in controlling and eradicating brucellosis reflect from avarn agement conditions (extensive breeding, trans humans, coexistence of several live stock species ,etc.). Most often, endemic are as are in countries with marked structural weaknesses ,an aggravating circumstance since efficient use of current vaccines requires proficient veterinary services. This requirement relates in part to some of the limitations of currently available brucellosis vaccines, and it seems likely that a perfect vaccine could greatly facilitate control and eradication. Godfroid **,** et al (2011).

The perfect brucellosis vaccine should: (i),trigger a solid and life lasting immunity; (ii) ,protect against infection by *Brucella* species other than those typical of a given host; (iii),be in nocuous regardless of the physiological state of the animal; (vi),be effective in a single dose;(v),not interfere with serological diagnostic tests; (iv),not be virulent for humans or carry resistance to antibiotics; (iiv),not be shed in the (environment be stable; and be affordable. Indeed, some of these requirements have become apparent only after using the classical brucellosis vaccines for more than half a century. those requirements have been met and the approaches followed to solve some of the problems. Researchers working on brucellosis vaccines in the past century progressively realized that killed vaccines were inferior to attenuated ones. Moreover, work with streptomycin-dependent *B. melitensis* mutants established that the ability to multiply in the host before clearance was a condition necessary to trigger protective immunity. Indeed, the two best vaccines developed *B. a bortus*

S19 and *B. melitensis* Rev 1are both attenuated (live)vaccines with a certain degree of residual virulence. Strain19 is used in cattle and Rev1 in goats and sheep, not only against *B. melitensis* but also against *B. ovis* in the latter ruminants.S19 is the result of accidental attenuation caused by prolonged laboratory storage under inadequate conditions (Nicoletti,1990) and carries multiple genetic defects which, in most cases, are difficult to relate to attenuation (Crasta *et al.*,2008). Rev1 is are vertant of a streptomycin-dependent mutant with a known ribosomal mutation (Cloeckaert *et al.*,2002) and possibly other genetic defects .Both vaccines carry a smooth(S) lipo polysaccharide (S-LPS)with an O-polysaccharide similar to that of the wild type *brucellae.* In controlled experiments ,a single dose of Rev1 induces 80–100% protection against challenges infecting100% of unvaccinated controls It has a low cost (5D cents per dose when applied conjunctivally).Limitations of this vaccine are the a bortifacient effect if applied during pregnancy, interference in serological diagnosis, virulence for human and resistance to streptomycin and tendency to dissociate into in effective rough [R] mutants. These limitations can be partially overcome by vaccinating animals conjunctively when they are less than 4months old which reduces greatly the interference in serological diagnosis and avoids vaccine-induced abortions; a minimal personal protection makes Rev1 vaccination safe ;and there are well-established quality control protocols. Rev1 has been crucial wherever *Br. melitensis* eradication has been achieved and, moreover, vaccination with Rev1 is economically sound (Zinsstag *et al.*,2007). Since cattle may become infected by *B. melitensis* (and by some *B. suis* biovars), it has been suggested that Rev1 could be used in the serum in ants. However, the protective efficacy against B. melitensis, innocuousness and safety of Rev1

in cattle is not known. B. melitensis infections in cattle can be controlled with the help of S19 but there is a paucity of studies with regard to B. suis. With the exception of a handful of countries with favorable geographical and management conditions ,all successful programs in cattle have used S19. In controlled experiments, the rate of protection is adequate in most cases (Nicoletti , 1990). Like Rev1in sheep, the choice of the vaccination route and vaccine dose is of paramount importance. Subcutaneously, standard S19 doses generate immune responses interfering in diagnostic tests and may induce abortions if applied during pregnancy and genital lesions in males. Moreover, a small proportion of animals may develop subclinical infections and shed the vaccine. Conjunctival vaccination with reduced doses when animals are less than 4months old avoid the abortions as well as the serological interference and udder infections. It is not known whether this route and doses makeS19 safe in males, appoint that would be worth investigating. Conjunctival vaccination is also adequate for vaccinating adult cattle since abortions and milk shedding are reduced to less than1%.This vaccine is very economical (about7D cents per individual dose), carries no antibiotic resistance and, although less virulent for humans than Rev1,also requires a minimal individual protection. Quality controls necessary and there are well-established protocols for this. Despite their limitations, S19 and Rev1 have been successfully used in some developed countries to eradicate brucellosis. However, their use in eradication programs poses the problem of distinguishing infected from vaccinated animals in serological tests. Although it is important to stress that this problem is of little or no significance in countries unable to implement testing and slaughtering programs, this has been considered the major drawback of these vaccines. Godfroid **,** et al (2011).

1-1-10. Bio-and agro terrorism :-

Brucella are highly potent pathogens in man and animals and as such also very effective biological agents for use in biological weapons. A low infectious dose of 10 bacteria and the fact that *brucella* are easily transmitted to humans via aerosols make these bacteria most attractive for military researchers (Hoover and Friedlander,2010). Consequently *B. suis* was one of the first agents being weaponries' (Pappas *et al.*,2006b). International disarmament efforts made it unlikely that biological weapons of mass destruction will be used by states' armies in modern wars now a days. However, fear shave arisen that biological weapons may be used against civilian gets by private organizations, groups or even individuals in attacks of bioterrorists. Rotz *et al*.(2002) made a risk assessment for a brucellosis outbreak with special respect on its influence on public health and medical infrastructure on a large scale using following criteria: public health impact, delivery potential to large populations, public perception, i.e., public fear and civil disruption, and special public health preparedness needs(Rotz etal.,2002). Brucellosis was ranked in the category B having only a lower medical and public impact(Pappas *et al*.,2006b;Rotz *et al*.,2002). It can be supposed that in countries which have successfully eradicated brucellosis, the first responders, e.g., family doctors, will not be aware of the clinical picture of the disease and that a delay in the diagnosis of the disease will result in a higher number off at al courses. Consequently, public health (i.e., medical awareness, surveillance and laboratory diagnostic capabilities)has to be strengthened in the future in both developed and developing countries. In the field of veterinary public health, the danger arising from agroterroism (the deliberate tampering with and/or

contamination of the food supply with the intent of adversely affecting the social, economic, physical, and psychological wellbeing of society)is of concern(Gyles, 2010). Targets may be farm animals(cattle, swine, sheep, horses, poultry and fish),field crops, processed food and storage facilities Countries being free from animal brucellosis may suffer from severe economic losses by the deliberate introduction of brucellosis into their bovine, caprine /ovine or porcine livestock .An attack with *Brucella* spp. May also be associated with severe outbreaks in the human population (Gyles, 2010).

1-1-11. Human brucellosis :-

Currently, only three no men species of the genus *Brucella* have an essential impact on public health, i.e., in order of their significance *B. melitensis*, *B. abortus*, and *B. suis*. Although human brucellosis is the most common bacterial zoonotic infection worldwide it is still are gionally neglected disease (Pappas *etal.*,2006b). The source of naturally acquired brucellosis in humans is almost always to be found in the animal reservoirs ,although very few cases of human to human transmission have been reported (Godfroid *et al.,* 2005).Human brucellosis is known to be highly endemic in the Mediterranean bas in, Middle East, Western Asia, Africa, and South America (Pappas *et al.*,2006b). Although animal brucellosis has been brought under control in several industrialized countries, human brucellosis occurs sporadically in individuals who acquire the infection abroad or by illegally imported ingestion of unsafe animal products and in occupationally exposed groups (Al Dahouk *et al.*,2005b). In 2008, a total of 619 confirmed human brucellosis cases were reported in the European Union the highest incidence was recorded in those member states not

officially free from bovine and ovine /caprine brucellosis (Greece, Italy, Portugal and Spain). At EU level, a statistically significant decreasing trend was observed during the five-year period 2004–2008. The peak of reported cases was observed in spring and summer (European FoodSafetyAgency,2010b). Childhood brucellosis *(B. melitensis)* in the United States is now an imported disease, primarily from Mexico. A study in Tanzania showed that medical professionals, especially those in rural areas had poor know ledge of zoonotic diseases (John *et al.*,2008). In areas where *B. abortus* is a major problem in cattle, seroprevalence rates in humans are estimated to be in the range of 1–5%(Swai and Schoonman, 2009) but in areas where *B. melitensis* is endemic (mainly in the middle East) higher prevalence rates have to be expected (Pappas *et al.*,2006b). The countries with the highest incidence of human brucellosis are Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman (Pappas *et al.*,2006b).

1.1.11-1. 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 thought 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).

The commonest mode of transmission is skinning stillborn lambs and kids and aborted foetuses, which may be heavily contaminated with *Brucella spp.*, and 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).). Consumption of raw milk continue to be the major mode of exposure in developing countries (Makita et al.,2008; Pappas et al.,2006b; Swai and Schoonman,2009). Although *Brucella* can be transmitted directly and indirectly from its animal reservoir to humans, indirect transmission remains the highest overall risk and mainly occurs through the consumption of unpasteurized milk or dairy products (Godfroid et al.,2005*). B. melitensis* infection in cattle has emerged as a serious public health problem in some southern European countries and Israel as a result of the consumption of unpasteurized milk since *B. melitensis* is capable of colonizing the bovine udder Lamontagne et al., (2010). Moreover, in some South American countries, cattle are now believed to be more important than pigs as a source of *B. suis* biovar 1infection for humans, because *B. suis* biovar 1 is capable of colonizing the bovine udder as *B. melitensis* does .Consequently ,human brucellosis is main l food-borne but can also be an occupational infection .The incubation period of the disease varies greatly, ranging from weeks to months.(Lamontagne et al., 2010). In addition, laboratory-acquired *Brucella* infection due to accidental ingestion, inhalation and mucosal 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). Studies conducted in North

Africa and in the Middle- East reported the occurrence of human brucellosis attributed to the presence of *B. melitenis* in livestock(Jennings etal.,2007) while in sub-Saharan African *B. abortus* is mainly implicated (Swai and Schoonman,2009).

1.1.11.2. 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. (Swai and Schoonman,2009).

1-1-11-3.Clinical manifestations :

The clinical onset of human brucellosis is insidious and *Brucella* infections often develop as fever of unknown origin. The acute stage of the disease is usually accompanied by bacteremia and spreading of the organism to various organ systems ,mainly to reticuloendothelial tissues .Hence, human brucellosis is a systemic infectious disease of varying clinical manifestations. Acute brucellosis is characterized by nonspecific systemic signs and clinical symptoms consistent with a flu-like or septicemia illness ,i.e., fever, fatigue, malaise, weight loss, headaches, arthralgia, myalgia, chills, and sweats. Clinical manifestations may comprise osteoarticular,

dermal,gastrointestinal, respiratory, cardiovascular , and neurologic disorders mimicking many other infectious and non-infectious diseases. Since *Brucella* survives and replicates in the mononuclear phagocytic system, chronic courses, focal complications, and relapses frequently occur. Life-threatening focal complications are *Brucella* endocarditis and neuro brucellosis but the overall case fatality rate is low (lessthan1%).(Godforid 2011)..

1-1-12. Bovine Brucellosis:

Bovine brucellosis is usually caused by *Brucella abortus, less* frequently by *brucella melitensis* and rarely by *Brucella suis*. It is characterized by abortion, with excretion of the organisms in uterine discharge and in milk. Major economic losses result from abortion. Loss of calves, reduced milk yield in females and infertility in males (WHO.1971 ,Radositis et al 2007).

production has been described in many developing countries as seen by the number of reports generated in the past 10 years. Cattle seroprevalence estimates have been observed to range between 3 and 15% Haileselassie et al.,(2010).Factors influencing prevalence include production systems, agro-ecological zones, husbandry practices, contact with wildlife, management factor (Matope et al., (2010). Abortion rated up to 50% Shepherd.

1-1-12-1. Transmission .

The infection is usually introduced into a herd through latently or acutely infected animals. The infection occurs mostly by ingestion of material which has been contaminated with the excretion of aborted female. The infection takes place through the mucosa even through respiratory system or the eye. Through injured or intact skin. Through mating (venereal disease) Insects may also carry the infection Large quantities of the bacteria are excreted with the fetus, the placenta and the uterine fluid, mainly at the time of calving. After abortion or parturition, the organism continues to be excreted mainly via the milk of infected cows According to infected breeding bulls can transmit the infection to cows at the time of service via the semen. Apart from direct contact between animals, other sources of infection within and between herds are contaminated water and feed supplies Kebeda et al (2008).

1-1-12-2.Pathogenesis:

Susceptibility of the cattle depends on the natural resistance, age, level of immunity and on environmental stress. If the infection is introduced into a non infected herds in which all animals are immunogenic ally naïve to brucellosis, storms of abortion occur and all pregnant cows will abort. After the infection of the regional lymph nodes, bacteraemia occurs and last for 1-3 weeks and distribute the organisms to lymphatic system, organs and tissues. In pregnant animals, the uterus is preferred site of infection which leads to necrotizing placentitis. In non pregnant animals, the first infection occurs in the udder followed by uterus infection after the onset of pregnancy.

The increased level of sugar Erythrol is enhanced the virulence of Brucella. (OIE 2009)

1-1-12-3. Clinical signs

Embryonal early death and thus symptomless infection.Abortion in the third of pregnancy. Abortion after the 7^{th} month of pregnancy. Birth of weak calves. Inflammation of the seminal vesicle and vesicular glands in bulls. Chronic inflammation of the epididymis in males, of the joints and the regional lymph nodes being enlarged and contain Brucella. After recovery, females are protected against renewed infection because of development of immunity. They are become fertile again or not because of permanent lesions in uterus. The incubation period varies between 14-120 days. If the infection is endemic , only the first calving animals abort. Before abortion, grey-whitish to reddish secretion appears in the vagina. Large amounts of pathogens are excreted with the lociae. Retained placenta is usually a consequence of abortion and can lead to permanent sterility. Infected bulls show an acute febrile general reaction, swollowen and painful scrotum, depression and inappetence. Hygromas especially in carpal joints, are characteristic feature of chronic infection. The most prominent clinical sign of bovine brucellosis is abortion or premature calving Other clinical signs are mainly the calving-associated problems and breeding-associated problems such as repeat breeding, a retained placenta and metritis .The infected cows usually abort only once after which a degree of immunity develops and the animals remain infected. At subsequent calvings, the previously infected cows excrete huge numbers of *Brucella* in the fetal fluids . (Ahmad et al 2009).

1-1-13.The epidemiology.

Several researchers have extensively reviewed the factors associated with *Brucella* infections of animals and they have classified each variable into one of three categories, which are related to the characteristics of the animal populations, the style of management and the biology of the disease. The factors influencing the epidemiology of brucellosis in cattle in any geographical region can be classified into factors associated with the transmission of the disease among herds and the factors influencing the maintenance and spread of infection within herds While trying to control or eradicate the infection, it is important to be able to separate these two groups of risk factors. The density of animal populations, the herd size, the type and breed of animal (dairy or beef), the type of husbandry system and other environmental factors are thought to be important determinants of the infection dynamics (Ahmad *et al* 2009).

In Sudan, Angara (2005) was studied the socioeconomic aspect of brucellosis in Kuku Dairy Scheme in 2004, the total cost of the disease in both dairy and health sectors was found to be 65833570 SD out of which 65617120 SD was the cost of the dairy sector and 216450SD was the cost of health sector. The burden of the disease was measured in disability adjusted life years(DALYs). In the year 2004, 7.1 and 14.1 years were lost if the disease is associated with level 0.1 and 0.2 disability weights, respectively.A seroprevalence study was conducted in Kuku Dairy Scheme, Khartoum North, Sudan. The scheme was proved to be endemic with bovine brucellosis. Cross-reaction with other bacteria and the possibility of false positive reactor animals due to vaccination had justified the use of competitive ELISA test for serum detection as a confirmatory test. The number of cattle examined, throughout the study, was 574 out of 845 cows

kept in Kuku Dairy Scheme. All the obtained sera were screened using Rose Bengal Plate Test (RBPT). Twenty eight out of the thirty herds of the sample had at least one positive reactor, resulting in 93.3% herd prevalence rate. All sera positive to Rose Bengal Plate Test (n = 178) were subjected to further confirmatory test using Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA). 143 cows (80.3%) were confirmed positive by c-ELISA. Out of 28 positive herds, 27 (96%) had at least one positive reactor. According to the confirmatory test, the herd prevalence rate was 90%, individual animal prevalence rate was 24.9% and average within herd prevalence rate was 24.5% (±15.7, CI 4.088 at 95%). The number of seropositive aborts was found to be 17 cows out of 143 (12%). It is concluded that bovine brucellosis was highly prevalent in Kuku Dairy Scheme. This fact justifies immediate adoption of an effective controlpolicy for this zoonotic disease. Angara (2005).

A serological study was carried out in Tiaret province in western Algeria on 1032 cows distributed in 95 flocks to estimate the prevalence of *Brucella* infection and to compare the sensitivityand specificity of a range of agglutination tests. Screening tests showed 31.5% of herds positive using the buffered plate antigen test and 26.3% using the rose Bengal test compared with 15.7% with the complement fixation test. Using the complement fixation test as the gold standard for confirmatory tests, the Rivanol test was found to be more sensitive but less specific than tube agglutination in detecting brucellosis infection. Three isolates were identified from 105 blood samples from humans with brucellos and 50 samples of milk and tissues from infected cows and they were all *Brucella melitensis* biovar 3. Aggad *et al*,(2006).

In Zambia cross-sectional study was investigated risk factors of *Brucella* seropositivity in cattle herds reared in livestock–wildlife interface areas of Blue Lagoon and Lochinvar National Parks in Zambia between August 2003 and September 2004. Sera were collected from cattle aged _2 years from 124 herds. Data on husbandry practices, grazing strategies, and herd structure (sex and age composition) were also collected. Sera were screened for anti-*Brucella* antibodies using the Rose Bengal test (RBT) as a presumptive test and a competitive-ELISA (c-ELISA) as a confirmatory test. Aherd was classified as *Brucella* seropositive if at least one animal tested positive on both RBT and c-ELISA in series testing. Risk factors for herd-level brucellosis seropositivity were tested using multivariable logistic regression; risk factors for increases in the within-herd counts of seropositive cattle were analyzed using the negative binomial regression model with the number of seropositive animals as outcome and total number of cattle tested in a herd as the population at risk (exposure). Of the 110 herds tested, 68 (62; 95% CI: 53, 71% after adjusting for clustering by area) tested seropositive for exposure to *Brucella* spp. The final logistic-regression model identified geographical area, with Lochinvar (OR = 3.4; CI: 0.97, 12) and Kazungula (OR = 4.3; CI: 0.91, 20) recording higher odds of *Brucella* infections compared to Blue Lagoon. Herds coming in contact with wildlife had higher odds compared to those without contact (OR = 3.4; CI: 1, 11). Similarly, the odds of *Brucella* infection were progressively higher in the larger herd categories (26–40 cattle, OR = 2.6; CI: 0.70, 10; 41–82 cattle, OR = 4.9; CI: 0.93, 26; >82 cattle, OR = 9.4; CI: 1.7–51) compared to the smallest herd category (10–25). The negative binomial regression model identified geographical area, contact with wildlife, and

herd size as having significant effect on counts of seropositive cattle in a herd. J.B. Muma et al (2007).

In Tigray Region Gebretsadik et al (2011).They were studied 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 from 816 extensively managed cattle herds above 6 months of age were screened for *Brucella* antibodies by the Rose Bengal Plate Test and reactosera were further tested by the Complemen 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. The results of univariate logistic regression analysis revealed that seropositivity to brucellosis was significantly higher in animals kept under the transhumance management system than animals in the sedentary system (P <0.001). The results also indicated that there was a statistically significant increase in seroprevalence to brucellosis with increasing age (P < 0.01) but not parity (P > 0.05). Significant increment of seropositivity was also observed as herd size increases from small to medium (P < 0.05) and then to large sizes (P < 0.001). In addition, a significantly higher seroprevalence was found in animals in the lowland than those in the highland agro-climatic zones. Nevertheless, in the multivariate logistic regression analysis, systemic factor (odds ratio [OR] = 10.6%, 95% confidence interval [CI] = 2.3-49.3, P < 0.01) and age (OR =

4.2, 95% CI = 2.3-49.3, $P < 0.01$) were identified as the major risk factors for individual animal seroprevalence. Furthermore, Fisher's Exact Test revealed that seropositivity to brucellosis had statistically significant association with history of previous abortions and stillbirths. The results of this study showed that brucellosis is an endemic and widely distributed disease in Tigray Region.

Ahmad et al (2009) were investigated the seroprevalence and risk factors for *Brucella* seropositivity in cattle in Jordan. The sera from 671 cows were randomly collected from 62 herds. The antibodies against *Brucella* were detected using a Rose Bengal plate test and indirect ELISA. A structured questionnaire was used to collect information on the cattle herds' health and management. A multiple logistic regression model was constructed to identify the risk factors for *Brucella* seropositivity. The true prevalence of antibodies against *Brucella* in individual cows and cattle herds was 6.5% and 23%, respectively. The seroprevalence of brucellosis in cows older than 4 years of age was significantly higher than that in the younger cows. The seroprevalence of brucellosis in cows located in the Mafraq, Zarqa and Ma'an governorates was significantly higher than that of the other studied governorates. The multiple logistic regression model revealed that a larger herd size (odd ratio <OR> = 1.3; 95% CI: 1.1, 2.6) and mixed farming (OR = 2.0; 95% CI: 1.7, 3.7) were risk factors for cattle seropositivity to *Brucella* antigens. On the other hand, the use of disinfectants (OR = 1.9; 95% CI: 1.1, 2.1) and the presence of adequate veterinary services (OR = 1.6; 95% CI: 1.2, 3.2) were identified as protective factors.

In Tanzania A cross-sectional epidemiological study was conducted to determine the seroprevalence and to identify risk factors for bovine

brucellosis seropositivity in traditional and smallholder dairy cattle production systems in the Tanga region of North-eastern Tanzania. The study populations comprised 246 indigenous and 409 crossbred cattle, randomly selected from 105 smallholder dairy and 25 traditional managed herds, respectively. Individual animal and herd-level data were collected using a structured questionnaire. Serum samples were screened for *Brucella* antibodies using the Rose Bengal Plate Test The overall seroprevalence of *Brucella* antibodies in the smallholder dairy and traditional managed cattle was 4.1% and 7.3% respectively. The corresponding overall herd prevalence was 10.5% and 20% respectively. Using multivariate logistic regression analysis, closeness to stock route, access to surface drinking water and location were identified as the major risk factors for individual herd seroprevalence. Older animals (≥6 years) were associated with increased risk of seropositivity compared to animals of age category of ≤6 years. The results showed that brucellosis is prevalent and widely distributed locally, underscoring the need for further studies including surveillance and institution of preventive and control measures particularly among female young-stock and the general public who are at high risk of contracting brucellosis. Emanuel et al(2010).

Kaoud et al (2010) were studied the epidemiology and the role of risk factors of *Brucella* infection in ruminants, besides the methods concerning the evaluation of biosecurity measures which are taken against the disease in farms. 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.

In Uganda . Human brucellosis has been found to be prevalent in the urban areas of Kampala, the capital city of Uganda . A cross-sectional study was designed to generate precise information on the prevalence of brucellosis in cattle and risk factors for the disease in its urban and peri-urban dairy farming systems. The adjusted herd prevalence of brucellosis was 6.5% (11/177, 95% CI: 3.6%-10.0%) and the adjusted individual animal prevalence was 5.0% (21/423, 95% CI: 2.7% - 9.3%) for *Brucella abortus* antibodies. Mean within-herd prevalence was found to be 25.9% (95% CI: 9.7% - 53.1%) and brucellosis prevalence in an infected herd ranged from 9.1% to 50%. A risk factor could not be identified at the animal level but two risk factors were identified at the herd level: large herd size and history of abortion. The mean number of milking cows in a free-grazing herd (5.0) was significantly larger than a herd with a movement restricted (1.7, $p < 0.001$ Makita et al; (2008) .

Bekele ,et al (2011).Found that bovine brucellosis has significant economic and zoonotic implication for the rural communities in Ethiopia in consequence of their traditional life styles, feeding habits and disease patterns. Hence, knowledge of brucellosis occurrence in traditional livestock husbandry practice has considerable importance in reducing the economic and public health impacts of the disease. A total of 1623 cattle sera were serially tested using the rose Bengal test as screening and complement fixation test. The Stata survey command was used to establish prevalence's 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.

A cross-sectional study was carried out on bovine brucellosis in Addis Ababa dairy farms from November 2003 to April 2004. A total of 1,202 blood samples were collected from non-vaccinated, cross-bred dairy cattle. The Rose Bengal plate test (RBPT) was used as a screening test. Those serum samples reacting positively to RBPT were subjected to the complement fixation test (CFT) for confirmation. The RBPT detected 30 of 1,202 (2.5%) of the samples as brucellosis positive. The positive sera when further retested using CFT, 18 out of the 30 RBPT positive sera were found to be positive. The prevalence of brucellosis based on CFT in the study area was 1.5%, and all positive sera were from female cattle.

Result of the questionnaire survey revealed that percentage of 4.4% abortion and 9.5% retained fetal membranes. Abortion and retained fetal membranes were associated with *Brucella* antibodies ($P<0.05$). A total of 153 cattle

attendants and owners in the farms were interviewed, and 73.5% were found to have no knowledge of brucellosis, only 20.8% wear protective gloves during handling aborted material and 39.6% responded that they consume raw milk. Results of this study showed that prevalence of bovine brucellosis in the study area is low and a test-and-slaughter policy can be used in order to control the diseases in dairy farms of Addis Ababa. Gebreyohans 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. 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 herd-level (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).

A cross-sectional study was performed in Southern and Lusaka provinces of Zambia between March and September 2008 to estimate *Brucella* seroprevalence in cattle kept by smallholder dairy farmers (n=185). Rose Bengal test (RBT) was used as a screening test followed by confirmation with competitive ELISA (c-ELISA). The investigated 1,323 cattle, of which 383 had a history of receiving wee used bovine vaccine and 36 had a

history of abortion. Overall seroprevalence was 6.0% with areas where vaccination was practiced having low seroprevalence. Age was associated with *Brucella* seropositivity (P=0.03) unlike cattle breed (P=0.21) and sex (P=0.32). At area level, there was a negative correlation (Corr. Co eff=−0.74) between percentage of animals with brucellosis vaccination history (vaccination coverage) and level of brucellosis; percentage of animals with history of abortion (Corr. Co eff.=−0.82) and brucellosis vaccination coverage. However, a positive correlation existed between brucellosis infection levels with percentage of animals having a history of abortion (Corr. coeff. = 0.72). History of vaccination against brucellosis was positively associated with a positive *Brucella* result on RBT (P=0.004) whereby animals with history of vaccination against brucellosis were more likely to give a positive RBT test results (OR=1.52). However, the results of c-ELISA were independent of history of *Brucella* vaccination (P=0.149) but was positively associated with history of abortion (OR=4.12). Our results indicate a relatively low *Brucella* seroprevalence in cattle from smallholder dairy farmers and that vaccination was effective in reducing cases of *Brucella* infections and *Brucella*-related abortions. John et al (2011).

IN Pakistan a total of 200 milk samples from cattle and buffaloes were evaluated using milk ring test (MRT) and indirect enzyme-linked immunosorbent assay (i-ELISA). The overall prevalence was found to be 3% and 8.5% in cattle and buffaloes using MRT and i-ELISA, respectively. The prevalence was 4.6% and 1.7% in cattle and buffalo using MRT, respectively, while i-ELISA exhibited 20% and 0% in cattle and buffalo, respectively. The prevalence was higher in government dairy farm, compared to privately owned dairy farm. This paper points out an alarming

situation in the target area with respect to the public health significance. Muhammad et al (2011).

In Nigeria a study for bovine brucellosis was conducted using serology to determine the status of the disease in slaughtered cattle. The sera were tested ELISA kits. An overall prevalence of 20% (64 positive) was obtained with sex prevalence for males and females being 10.62% (34 positive) and 9.37% (30 positive) respectively out of 180 males and 140 females tested without significant association ($P < 0.05$). On age distribution, higher prevalence of 11.87% was recorded in age group > 24 months while; lower prevalence of 3.13% was recorded in age group < 12 months. There was no significant association statistically between age and occurrence of brucellosis. White Fulani breed had the highest prevalence of 8.75%. There was significant association ct

This study was carried out to investigate the status of brucellosis in cattle under various management systems Adamawa, Kaduna and Kano states, northern Nigeria . Using multi-stag sampling, serum samples of 4,745 cattle from 271 herds were tested using the Rose-Bengal plate-agglutination test (RBPT) and positives sera were confirmed using a c-ELISA. The Results: Prevalence estimates were calculated by adjusting for sampling weights and where possible for test sensitivity and specificity. Showing 37% RBPT positive, and after confirmation with c-ELISA the overall animal-level prevalence, adjusted for sampling weights, was 26.3% (95% CI, 22.1%-31.0%). Of the herds sampled, 210 (77.5%; 95% CI, 68.6%-84.5%) had at least one animal positive to both tests; showing no defenses between states ($P = 0.538$). Mean within-herd seroprevalence in positive herds was 30.2% (95% CI, 25.3%-35.1%) and ranged from 3.1% to 85.7%. Overall animal-level seroprevalences of 29.2% (95% CI, 22.5%-36.9%) n =

1,827, 23.3% (95% CI, 18.9%-28.3%) n = 1,870 and 26.7% (95% CI, 18.8%-36.7%) n = 1,048 were observed in Adamawa, Kaduna and Kano states, respectively (P = 0.496). A significantly higher seroprevalence was found in males (38.2%; 95% CI, 31.7%-45.2%) than in females (24.7%; 95% CI, 20.4%-29.5%) (P < 0.001) and in non-pregnant females (27.8%; 95% CI, 22.9%-33.5%) than in pregnant females (17.2%; 95% CI, 13.6%-21.5%) (P < 0.001). Seroprevalence increased with increasing age (P < 0.001), from 13.5% (95% CI, 8.9%-19.9%) in cattle <4 years to 35.0% (95% CI, 28.5%-42.3%) in cattle >7 years. Seroprevalence also varied between management systems (P < 0.001): pastoral systems 45.1% (95% CI, 38.6%-51.9%), zero-grazing systems 23.8% (95% CI, 6.8%-59.2%), agro-pastoral systems 22.0% (95% CI, 17.3%-27.8%), and commercial farms 15.9% (95% CI, 9.5%-25.5%). Seroprevalence did not differ significantly between breeds or lactation status. The pastoral management systems of the traditional Fulanis may be encouraging the dissemination of the disease. Public enlightenment of the farmers about the disease, vaccination and appropriate national control measures are recommended. statistically ($p < 0.05$) between breed and infection. Lawala et al (2012).

In Nigeria Mai et al (2012) were investigated the status of brucellosis in cattle under various management systems Adamawa, Kaduna and Kano states, northern Nigeria. Using multi-stag sampling, serum samples of 4,745 cattle from 271 herds were tested using the Rose-Bengal plate-agglutination test (RBPT) and the positives sera were ivestegated by(c-ELISA).Results: Prevalence estimates were calculated by adjusting for sampling weights and where possible for test sensitivity and specificity. 37% of all animals were RBPT positive, and after confirmation with c-ELISA the overall animal-level prevalence, adjusted for sampling weights,

was 26.3% (95% CI, 22.1%-31.0%). Of the herds sampled, 210 (77.5%; 95% CI, 68.6%-84.5%) had at least one animal positive to both tests; this did not differ significantly between states (P = 0.538). Mean within-herd seroprevalence in positive herds was 30.2% (95% CI, 25.3%-35.1%) and ranged from 3.1% to 85.7%. Overall animal-level seroprevalences of 29.2% (95% CI, 22.5%-36.9%) n = 1,827, 23.3% (95% CI, 18.9%-28.3%) n = 1,870 and 26.7% (95% CI, 18.8%-36.7%) n = 1,048 were observed in Adamawa, Kaduna and Kano states, respectively (P = 0.496). A significantly higher seroprevalence was found in males (38.2%; 95% CI, 31.7%-45.2%) than in females (24.7%; 95% CI, 20.4%-29.5%) (P < 0.001) and in non-pregnant females (27.8%; 95% CI, 22.9%-33.5%) than in pregnant females (17.2%; 95% CI, 13.6%-21.5%) (P < 0.001). Seroprevalence increased with increasing age (P < 0.001), from 13.5% (95% CI, 8.9%-19.9%) in cattle <4 years to 35.0% (95% CI, 28.5%-42.3%) in cattle >7 years. Seroprevalence also varied between management systems (P < 0.001): pastoral systems 45.1% (95% CI, 38.6%-51.9%), zero-grazing systems 23.8% (95% CI, 6.8%-59.2%), agro-pastoral systems 22.0% (95% CI, 17.3%-27.8%), and commercial farms 15.9% (95% CI, 9.5%-25.5%). Seroprevalence did not differ significantly between breeds or lactation status. This is the first large study to assess the prevalence of bovine brucellosis over a wide geographic area of northern Nigeria, in a variety of management systems and using accurate tests. The seroprevalence of brucellosis was high, and higher than results of previous studies in northern Nigeria. The pastoral management systems of the traditional Fulanis may be encouraging the dissemination of the disease. Public enlightenment of the farmers about the disease, vaccination and appropriate national control measures are recommended.

Abdalla et al (2012)were studid to detect brucellosis in suspected dairy cattle in Khartoum State, Sudan using the conventional serological tests and tests done on milk in comparison to a PCR-based technique. Milk and blood samples collected simultaneously from suspected brucellosis cows (n = 147) in 12 different dairy farms around Khartoum State were used in the study. Overall, 54 (36.7%) of the total milk samples were positive according to the milk ring test (MRT), while 29 (19.7%) of the serum samples were positive according to the Rose Bengal test (RBT); microscopy on modified Ziehl–Neelsen-stained slides detected 13.6% of the cases, and recovery of *Brucella* species on both *Brucella* medium and tryptic soya agar was 7.5%. Thirty-three (22.4%) samples were found positive on PCR-amplified IS711 which were then taken as positive brucellosis cases. The differences of RBT and PCR-IS711 from MRT were highly significant ($P < 0.05$). MRT detected more cases of bovine brucellosis compared to RBT, PCR, microscopy, and culture. MRT is recommended as a noninvasive test compared to RBT, and it is less expensive compared to PCR and culture.

Mahmoud et al (2012) were determined the prevalence of the brucellosis in cattle in Eldein area, Eastern Darfur state, Western Sudan. Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Competitive Enzyme Linked Immuno Sorbent Assay (cELISA) were used for the diagnosis of the disease. RBPT showed 21 (8.4%) positive results, SAT showed 50(20%) positive sample and cELISA showed 5(2%) positive samples. Twenty four (9.6%) cows had a history of abortion, 20 (8%) had histories of retained placentas and 3 (1.2%) had knee hygromas. The study revealed that sex, age and breed were not associated with the brucella

seropositivity (P>0.05). In this study, three types of serological test were used, namely RBPT, SAT and cELISA and there was significant difference between any two given tests (P<0.05). Using agreement between tests (Kappa Statistic) indicated that RBPT and SAT had moderate agreement (Kappa=0.547), RBPT and cELISA had fair agreement (Kappa=0.364) and SAT and cELISA had slight agreement (Kappa=0.158). cELISA is the most sensitive and reliable test in field .

Malaysia study was carried out to elucidate the seroprevalence of brucellosis in small and large ruminants and estimate the economic impact of zoonotic brucellosis in Malaysia using available data.

Data was collected during culling exercises by the Department of Veterinary Services of Malaysia as a result of surveillance using CFT. The average compensation in 4 years per district of Melaka state was RM12248.875(USD 3874.75) and the total compensation paid in 4 years was RM146,986.50(USD45,865.24) with year 2009 having the highest compensation amount of RM58,914.40(USD18,383.48). The estimated total economic losses due to brucellosis stands at about RM200,607,946.80 (USD 62,926,060.84) in a year for the whole of Malaysia. The odds of brucellosis in large ruminants (cattle/buffaloes) was significantly 1.6 times more compared to small ruminants (goats/sheep) in Melaka (P<0.0001; C.I. 1.41, 1.81) during the 4 year period. Average 4 year total seroprevalence for brucellosis in Melaka was significantly higher in 2010 than previous years with a rate of 7.78 % (P<0.05; Phi=0.025). Mass importation of livestock may be contributing in complicating the brucellosis situation. Considering the economic importance of brucellosis and its epidemiological importance

to public health more needs to be done to ensure successful eradication of the zoonotic disease in Malaysia Bamaiyi et al (2012) .

Maurice et al (2013) were determined the seroprevalence of brucellosis among cattle. Sera obtained from a total of 270 randomly selected cattle from different herds in the four selected LGAs were for Brucella antibodies using the Rose Bengal Plate Test (RBPT).: An overall brucellosis seroprevalence of 9.6% (26/270) was obtained. The seroprevalence of Brucella antibodies among the cattle . Also females had a higher percentage of seropositives compared to males while cattle reared under extensive system of management had a higher (11.6%) percentage of sero-positives compared to cattle kept under the intensive system of management. However, there was no statistically significant (P>0.05) association between serological status and sex or management.

Across-sectional study was conducted to determine the prevalence of bovine brucellosis in three cattle production systems in 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 2013)

Across-sectional study was carried out in 2009. in Eritrean dairy cattle, to get a reliable estimate of brucellosis prevalence. The survey considered the sub-population of dairy cattle reared in modern small- and medium-sized farms. Samples were screened with the Rose Bengal test (RBT) and positive cases were confirmed with the complement fixation test (CFT). A total of 2.77% (417/15 049; Credibility Interval CI: 2.52% – 3.05%) of the animals tested in this study were positive for antibodies to *Brucella* species, with a variable and generally low distribution of positive animals at regional level. The highest seroprevalence was found in the Maekel region (5.15%; CI: 4.58% – 5.80%), followed by the Debub (1.99%; CI: 1.59% – 2.50%) and Gash-Barka (1.71%; CI: 1.34% – 2.20%) regions. Seroprevalence at sub-regional levels was also generally low, except for two sub-regions of Debub and the sub-region Haicota from the Gash-Barka region. Seroprevalence was high and more uniformly distributed in the Maekel region, namely in the Asmara, Berik and Serejeka sub-regions. Considering the overall low brucellosis prevalence in the country, as identified by the present study, a brucellosis eradication programme for dairy farms using a test-and-slaughter policy would be possible. However, to encourage the voluntary participation of farmers to the programme and to raise their awareness of the risks related to the disease for animals and humans, an extensive public awareness campaign should be carefully considered, as well as strict and mandatory dairy movement control. (Scacchia et al 2013).

The sero-prevalence of brucellosis was investigated among different breeds of cattle and buffaloes in Pakistan. A total of 2330 milk samples (1168 cattle and 1162 buffaloes) were screened for the presence of *Brucella abortus* antibodies using the milk ring test (MRT). Information related to animal type, urbanicity, sampling area and breeds were collected with the help of a pretested questionnaire on the day of sampling. The overall sero-prevalence was 6.9% in cattle and 6.6% in buffaloes. The odds of brucellosis sero-positivity were higher among cross breed cattle and Nili-ravi buffaloes. This study is the first evidence of prevalence of *Brucella abortus* up to breed level in dairy cattle and buffaloes in Pakistan.(Ali et al (2013).

. Anka et al (2013**).** described the distribution, pattern and trend of bovine brucellosis in Peninsular Malaysia between 2000 and 2008 based on serological data obtained from nationwide *Br.abortus* serosurveillance activities in cattle populations. Brucella antibodies were detected in 21.8% of sampled herds (95% CI, 21.01–22.59) and 2.5% (95% CI; 2.45–2.55) of sampled cattle. The state of Pahang had the highest animal and herd-level seroprevalence of 5.3 and 43.6%, respectively. The herd-level seroprevalence varied but remained high (18-26%) over the period of study and generally increased between 2000 to 2008. Seropositive herds clustered around the central part of the peninsula within the period of the study. The months of September, October and November illustrated the highest rates with corresponding seroprevalences of 33.2, 38.4 and 33.9%, respectively. A noticeable variation was observed in the cattle-level seroprevalence, but the rate remained relatively low (<5%). The chi-square statistics showed herd size ($\chi2 = 1206.077$, df = 2, p = 0.001), breed ($\chi2 = 37.429$, df = 1, p = 0.001), month of sampling ($\chi2 = 51.596$, df = 11 p = 0.001), year ($\chi2 =$

40.08, df = 8, p = 0.001) and state ($\chi 2$ = 541.038, df = 10, p = 0.001) to be associated with increased seropositivity. Bovine brucellosis is widespread among herds in Peninsular Malaysia at a low within-herd seroprevalence rate.

A cross-sectional study was carried out in three livestock species (cattle, camels and goats).To assess seroprevalences of *Brucella* and *C. burnetii* in pastoral livestock in southeast Ethiopia, The study was conducted from July 2008 to August 2010, and eight pastoral associations (PAs) from the selected districts were included in the study. Atotal of 1830 animals, comprising 862 cattle, 458 camels and 510 goats were screened initially with Rose Bengal plate test (RBPT) for *Brucella*. All RBPT positive and 25% of randomly selected negative sera were further tested by ELISA. These comprise a total of 460 animals (211 cattle, 102 camels and 147 goats). Out of sera from total of 1830 animals, 20% were randomly selected (180 cattle, 90 camels and 98 goats) and tested for *C. burnetii* using ELISA. The seroprevalences of *Brucella* was 1.4% (95% confidence interval (CI), 0.8-2.6), 0.9% (95% CI, 0.3-2.7)b and 9.6% (95% CI, 5.2-17.1) in cattle, camels and goats, respectively. Goats and older animals were at higher risk of infection (OR=7.3, 95% CI, 2.8-19.1) and (OR=1.7 95% CI, 0.9-2.9), respectively. Out of 98 RBPT negative camel sera, 12.0% were positive for ELISA. The seroprevalences of *C. burnetii* were 31.6% (95% CI, 24.7-39.5), 90.0% (95% CI, 81.8-94.7) and 54.2% (95% CI, 46.1-62.1) in cattle, camels and goats, respectively. Gumi et al (2013).

In India study was investigated to record the seroprevalence of brucellosis in cattle and buffaloes, by employing the three serological tests viz. Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (SAT) and Indirect-Enzyme Linked Immunosorbent Assay (I-ELISA) and to compare

their sensitivity and specificity. The study also aimed to assess the therapeutic efficacy of combination of long acting oxytetracycline and streptomycin in brucellosis infected cattle. A total of 250 serum samples; 176 from cattle and 74 from buffaloes were screened for presence of *Brucella* antibodies by RBPT, SAT and Indirect ELISA. The overall seroprevalence of brucellosis in Chhattisgarh state of India by RBPT, SAT and I-ELISA was 13.0% 19.8% and 31.2% respectively in cattle whereas 16.2%, 14.8% and 20.2% respectively in buffaloes. Cattle of >6 years age group showed highest seroprevalence followed by 4-6 years
and lowest in 0-2 years age group. On the contrary, buffaloes of 4-6 years age group showed highest seroprevalence followed by >6 years age group. Seroprevalence was higher in crossbred than indigenous cattle and more in female animals in cattle and buffaloes. Sensitivity of RBPT and SAT was recorded 47.14% and 57.14%, while specificity was recorded 98.88% and 96.11% respectively. Thus, SAT was found to be more sensitive but less specific than RBPT. In this study, overall agreement of RBPT and SAT with ELISA was found to be 84.4% and 85.2% respectively. (Nitu et al 2013) **.**

Bovine brucellosis was investigated inTajikistan 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 found that 4.1 and 2.0 %, re spectively. 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. Elisabeth .et al (2014).

1-1-14- .Economic Impact of bovine brucellosis:-

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 *Brucella* infection in pregnant cows can cause abortion or premature calving. Furthermore, *brucella* infection can lead to temporary sterility death from acute metritis and decreased milk production In cattle, brucellosis continues to contribute to economic losses associated with abortions, infertility and prolonged calving to conception intervals. The odds of abortion in *Brucella* infected cattle have been observed to range between 3 and 4 in exposed cows compared to non-exposed (Muma *et al.*,2007a;) It is a zoonotic infection and a serious threat to public health. Losses due to abortion or still births, irregular breeding, loss of milk and meat production and infertility are substantial. Apart from the above, human sufferings and agony is enormous. The impact of the latter can hardly be measured in medical care alone as Godfroid , et al. (2011)**.** estimated each case at US$ 3200. They also concluded that infected non aborting dairy cows produced 10% below potential and abortions at 20%. They further estimated that 10-35% of infected cows abort each year.

General economic losses, however, go far beyond the financial losses suffered by cattle producers alone. Not only cattle but also other species might be affected by brucellosis, including humans. summarized the economic losses of brucellosis to be:

- Losses due to abortion in the affected animal population;
- Diminished milk production, *Brucella* mastitis and contamination of milk;
- Cull and condemnation of infected animals due to breeding failure;
- Endangering animal export trade of a nation;
- Human brucellosis causing reduced work capacity through sickness of the affected people; 6) Government costs on research and eradication schemes;
- Losses of financial investments. Most studies that focused on brucellosis in African cattle highlight the fact that the control of brucellosis is of economic importance. However, only very few studies were found to have carried out a crude economic analysis to evaluate the impact of bovine brucellosis in traditional cattle systems in SSA, or to evaluate the possible costs of controlling the disease, for example, conducted a preliminary evaluation of the possible costs and benefits to cattle farmers from controlling brucellosis Godfroid , et al. (2011).

In Central America during the last 10 years has been estimated as between 4 and 8%, with higher prevalence in dairy herds and with losses calculated at US$ 25 million per year (Moreno, 2002). In Ethiopia, information on losses specifically through brucellosis in the different types of production systems is sparse, with the exception of Tariku (1994) who reported an annual loss from brucellosis estimated to be 88,941.96 Ethiopian Birr ($5231equivalent) among 193 cattle, largely due to reduced milk production and abortions (Chaffa State Farm, Wollo, from 1987 to 1993).

The estimated total economic losses due to brucellosis stands at about RM200,607,946.80

(USD 62,926,060.84) in a year for the whole of Malaysia Pwaveno et al (2012) . In Turkey total financial losses caused by brucellosis, respectively in optimistic, expected and pessimistic scenarios, were calculated as $20.066.875, $41.337.446 and $61.711.571 Can et al (2011). In Sudan Anagra,(2005) estimated the cost of brucellosis in Kuku dairy fscheme , the total losses accounted to SD 66,910,503 equivalent to U$$ 267,642. In Jabl Aolia locality Khartoum State ,Sudan Osman (2015) estimated the cost of brucellosis , the total losses accounted to SD 3,402,620 equivalent 73, 970,0 U$$

Chapter Tow

Materials and methods

2-1 Study area :-

The study was conducted in Khartoum State localities dairy farms . Khartoum state is one of eighteen (18) State in Sudan, it consist of seven localities, Shargelnel; Bahry; Khartoum; Jablaolia ; Omdurman; Umbda and Karry. The study was conducted in the six localities, (Shargelnel; Bahry; Khartoum; ; Omdurman; Umbda and Karry.)

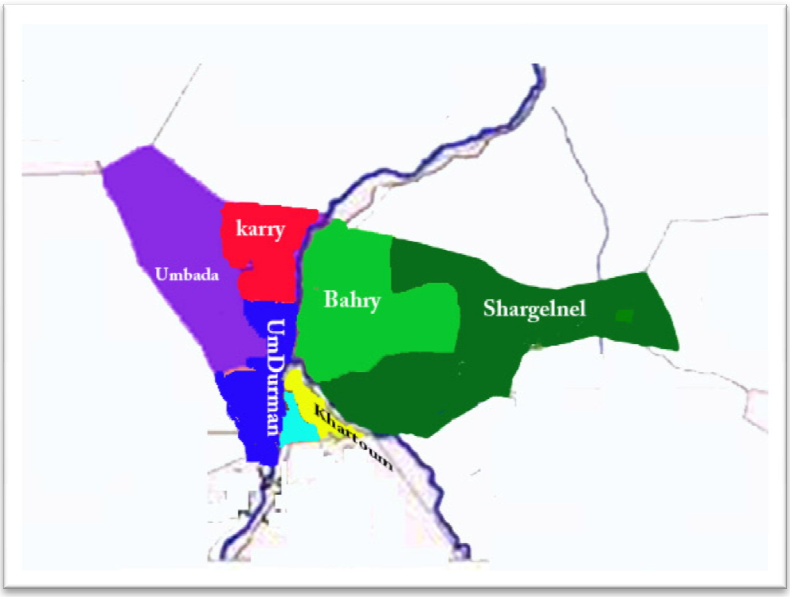

Figure (1) : Khartoum State localities

Khartoum State is located in central of Sudan with a population of 5.3 millions (National population 2008). Livestock population is constituted of 23995 head of cattle , 63416 head of goat, 504078 head of sheep and 6585 head of camel (Agricultural census 2008).

2-2 Study design :-

Across –sectional seroprevalence survey was conducted and structured questionnaire was designed to collect information on individual herds from the animal owners. Risk factors such as age, sex, herd size ,geography, history of abortion , history of vaccination, mixed farming, type breed, mixed age, calves bar, breeding methods (natural ,artificial) and presences of veterinary services , awareness, water supply were investigated. (Appendix,1)

2-3 The target population :-The animal used in the study were dairy cattle, which consist of breeding cows, replacement heifer and bulls.

2-4 Sampling Methods :

The blood samples were collected from cattle in six localities by multistage sampling method , stage 1 selecting (localities) ,stage 2 selecting simple random sampling (Farms ,one hander thirty three farms) and stage 3 then a sample of individual animals was selected by simple random sampling methods,

- Herd with number < 10 cows 10% were selected.

- Herd with number ≥10 cows all number were selected.

then a sample of individual animals was selected by simple random sampling methods (Martin *et al*. 1987).

2-5 Sample size determination and Sample collection :-

2-5.1 Sample size determination :-

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

$N= \quad 4\ pQ/L^2$

n = Required sample size ,

4 = Constant.

P = Expected prevalence = prevalence of each locality, shargelneel 22%, Bhary 33% , Khartoum 33% , Omdraman 17.6% , Karry 18.3% , Umbada 21.2 %. (Anoon 2011) .

Q = 1- prevalence

$L^2 = (0.05)^2$ allowable error.

$N = 4\ pQ\ /L^2$

N = 4x33x77/2 5 = 406 Bahry

N = 4x33x77/25 = 406 Khartoum

N = 4x17x83/2 5 = 225 Omdurman

N = 4x 22x88/25 = 309 Shargelnel

N = 4x 21x89/ 25 = 299 Umbada

N = 4x18 x 82/25 = 236 Karry

2-5-2 Sample collection :-

Blood samples were collected from the jugular vein of 1286 cattle of different ages and sex from 133 herds selected from six localities using vacutainer tubes and an identification code was given to the sample. Blood samples were centrifuged to allow serum separation . The sera were transported from the collection site to the Veterinary Laboratory using an ice-box and were be kept at -20$C°$ until analysis. The serum samples were tested using Rose-Bengal Test (RBT), and competitive Enzyme Linked Immuno-Sorbent Assay (cELISA) to determine the seroprevalence in the herd, locality, and the state.

2-6 Serological Analyses:-

2-6-1 Laboratory diagnosis:

The serum samples were centrifuged using a 3400/ minute centrifuge after kept at room temperature for 5minutes,then separated , preserved in 1.5 eppindorv tubes and refrigerated overnight for testing . The laboratory diagnosis rely mainly on serological tests namely Rose–Bengal Plate Test (RBPT) and Competitive Enzyme linked Immuno- Sorbent Assay (cELISA).

2-6-1-1 Rose –Bengal Plate Test (RBPT):-

The serum samples were first screened using Standardized buffered Rose – Bengal stained antigen (RBT) which were obtained from the Central Veterinary Laboratory Soba, Sudan. Then the positive samples were subjected to confirmatory test using Competitive Enzyme linked Immuno- sorbent Assay (cELISA).

2-6-1-1-1 Materials and reagents of Rose-Bengal Plate Test :-

-Rose Bengal antigen –Micropipette -Test plate –Shaker

-Mixing rods -Timer

2-6-1-1-2 Test procedure of Rose-Bengal Plate Test :-

Standardized buffered Rose Bengal stained antigen was used to screen all sera. An earlier described method was applied ,(Alton *et al.*, 1975). Briefly: equal quantities (0.03) ml of serum and buffered antigen were placed in a circle on test plate using micropipette, mixed, and spread. The tests were read immediately after 4-minute rocking period in room temperature and the degree of agglutination was read as positive.

2-6-2:Competitive Enzyme Linked Immuno –sorbent Assay (cELISA)

The RBPT positive sera were all tested using the Svanovir TM Brucella-Ab c-ELISA test kits (Svanova Biotech, Uppsala, Sweden) at NAHDIC. The test was conducted according to the instructions of manufacturer. The test was preformed in 96-well polystyrene plate (Nalge Nune, Denmark) that were pre-coated with *Brucella* species lipopolysaccharide (LPS) antigen. Serum diluted 1:10 was added to each well followed by equal volume of pre-diluted muse monoclonal antibodies specific for a common epitope of the O- polysaccharide of the smooth LPS molecule .The reactivity of the mouse monoclonal antibody was detected using goat antibody to mouse IgG that was conjugated to horseradish peroxidase .Hydrogen peroxidase substrate and ABTS chromagen were developed for 10 min. The reaction was then stopped using I M H2SO4. Optical densities were read at 450 nm using Titertek Multiscan® PLUS reader

(Flow Laboratories, UK). The threshold for determining , seropositivity was based upon the manufacturer´s recommendations (>30%), with antibody titer recorded as percentage inhibition as defined by the ELISA kit supplier.

2-7. Questionnaire Design and Data Collection

Information about each herd and the animals kept was collected by means of a structured questionnaire, which was completed at all the selected herds on a single visit. The questionnaire was designed to include important herd and animal level data these record included cattle location locality, herd type (one species, multi), sex (male, female),breeding method used (natural , artificial insemination), Bull (own bull , share). Breed (local. Cross) ,Source of drinking water (tap water, well, common canal) ,Vaccination (yes, no), History of abortion (yes ,no), Owner awareness (yes, no), Mix age (yes, no) ,Calves bar (yes, no), Housing (intensive ,semi intensive), Age (≥3 years, < years), Herd size (≥30, 30-60 , <60 cows). The economic significance of the disease with regard to production losses and negative impact, included the price of farm products, price of LB of milk, price of male calves, price of female calves , average milk production per day and the price of infected cows .

2.8 Data Management and Statistical Analysis

The questionnaire data were transferred into a Microsoft Excel spreadsheet. Descriptive statistics: the data collected from questionnaire survey were analyzed using descriptive statistical methods. Association between a potential risk factor and proportion of disease-free herds and infected herds

was expressed by the odds ratio (OR). Chi-square test was used for univariate analysis with p-value of 0.25 , and each factor with p-value equal or less than 0.25 was entered to multivariate analysis which was done by Logistic Regression and each factor in multivariate analysis with p-value less than or equal to 0.05 was considered statistically significant.

2.9. Analysis of the economic data :-

from the known methods of economic impact assessment of animal disease partial budgeting according to Morris (1999) was used to evaluate the economic impact of an endemic disease. in 6 localities Khartoum,Omdurman, Karry, ombada, Bahry ,Sherg elneel

2.9.1. Parameters used and their sources

2.9.1.1. Khartoum,locality

1. The total of mature cow in Khartoum ,locality= Agriculture census (2008):

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

3. The total number of mature cow sample = (the field survey)

The following parameters were estimated

4. Seroprevalence

5.. Abortion rate

6. Repeat breading rate

7. Reduction in milk production by 20% for aborted and 10%for non aborted (Zinsstag *et al* 2007).

8. Average annul milk yield (Medani,1996).

9. The average price of milk/L.

10. Price of female calve at weaning weight.

11. Price of male calve at weaning weight

12. Cost of repeat breeding

2.9.2. Calculation of economic loss of bovine brucellosis

2.9.2.1. calculation of economic loss of bovine brucellosis in selected sample.

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

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

Total quantity of milk lost = Quantity of milk loss of seropositive aborted animals+ Quantity of milk loss of seropositive none aborted animals.

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

Quantity of milk loss of seropositive none aborted animals= (Number of none aborted seropositive animals x average annual milk yield x 10%).
Value of milk lost = Total quantity of milk lost x price of milk...…..(1).

2.9.2.1. 2. Losses due to loss of aborted foeti:

Number of aborted foeti in the sample = Number of aborted seropositive cows. We supposed that 50% of the aborted foeti were females and the rest 50% were males.

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

2.9.2.1. 3. Loss due to repeat breeding :

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

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

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

2.9.2.2 Calculation of economic loss of bovine brucellosis in herd studied.

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

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

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

2.9.2.4 Calculation of economic loss of bovine brucellosis in the Khartoum state.

Total loss due to bovine brucellosis in six Khartoum state localities =

Khartoum + Omdurman + Karry + Umbada + Bahry +Sherg elneel.

Chapter Three

Results

3.1 Result of Serological Survey:-

3.1.1. Prevalence of bovine brucellosis among 1286 cattle examined by RBPT in Khartoum State:-

In the RBPT**,**105 out of the133 herds studied were seropositive. The overall herd prevalence brucellosis was78.9%. , i.e. at least one positive to RBPT identified in a herd. Within the herd prevalence ranged between 0% - 80%. Out of 1286 serum samples tested 332 were positive to Rose Bengal Plate Test, resulting in 25.8% individual animal prevalence (Table, 1).All sera positive to RBPT (332 samples) were subjected to further confirmatory test using competitive Enzyme Linked Immunosorbent Assay . (c-Elisa) from 332 samples 170 samples were confirmed (c-Elisa) positive.

Table 1: Prevalence of bovine brucellosis among 1286 cattle examined by RBPT in Khartoum State

	Frequency	Relative frequency	Cumulative frequency
Negative	954	74.2	74.2
Positive	332	25.8	100.0
Total	1286	100.0	

3.1 .2. Prevalence of bovine brucellosis among 1286 cattle examined by localities in Khartoum State :-Among the localities the rate of infection was high in the Khartoum locality 33.3%, followed by 30.8% in Umbada, 29.5% in Bahary , 23.1% in Omdurman,22.9 in Shargelneel and 9.2 % in Karry (Table, 3). The Chi-square test showed significant association between infection and localities (P – value = 0.000 ≤ 0.25), . (Table,4). (Fig2).

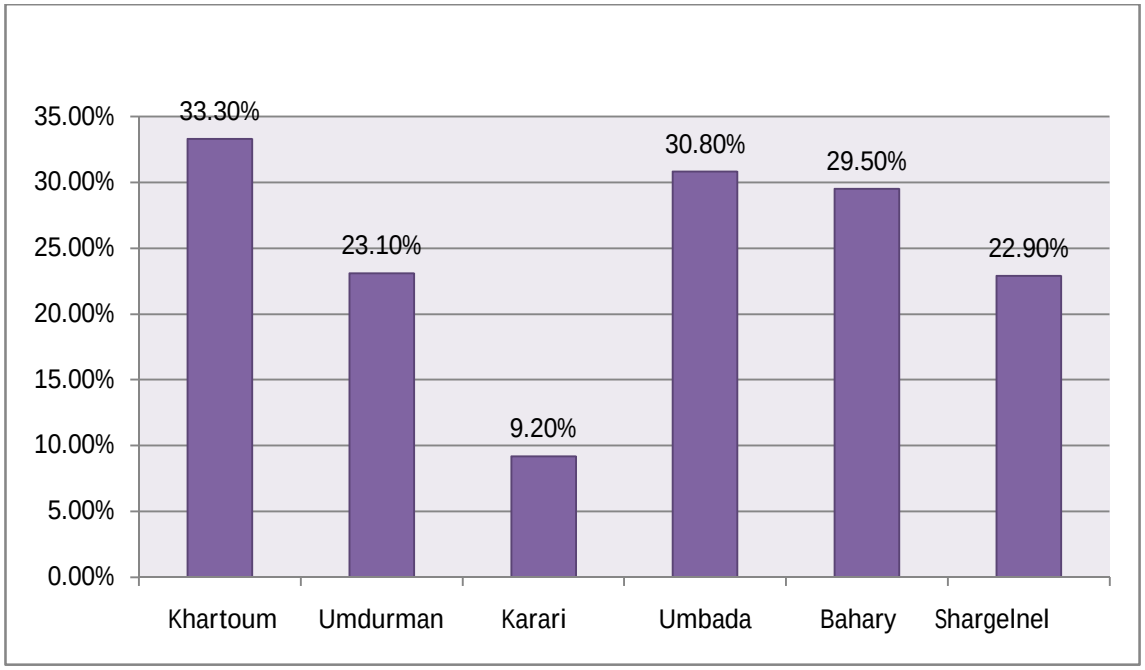

Fig2.Seroprevelance of bovine brucellosis in Khartoum State localities

3.1.3. Prevalence of bovine brucellosis among 1286 cattle examined by Herd size:-

A total of 1286 cattle of various Herd size were examined in this study. Table ,2 shows the Herd size distribution of cattle. The prevalence among the Herd size showed that cattle less than 30 had a prevalence of 26.1 %,

and cattle from 30-60 had a prevalence of 24.7% and cattle more than 60 had a prevalence of 26.2 (Table, 3). The Chi- square test showed no significant association between infection and Herd size of animal (p- value = 0.869) (Table, 4).

3.1.4. Prevalence of bovine brucellosis among 1286 cattle examined by Herd type:-

A total of 1286 cattle of various herd types were examined in this study. Table ,2 shows the Herd types distribution of cattle. 1039 of cattle were cattle only and 247 cattle were with mixed spices. The prevalence among the herd types showed that cattle only had a prevalence of 27.9 %, and cattle with mixed species were of prevalence of 17% (Table, 3). The Chi- square test showed significant association between infection and herd type of animal (p- value = 0.000) (Table, 4).

3.1.5. Prevalence of bovine brucellosis among 1286 cattle examined by Breed:-

A total of 1286 cattle of various breed were examined in this study. Table ,2 shows the breed distribution of cattle. 41of cattle were local breed and 1245 of cattle were cross breed. The prevalence among the breed showed that local breed had a prevalence of 26.4 %, and cross breed had prevalence of 7,3% (Table, 3). The Chi- square test showed significant association between infection and breed of animal (p- value = 0.060) (Table, 4).

3.1.6. Prevalence of bovine brucellosis among 1286 cattle examined by veterinary services:-

A total of 1286 cattle of various veterinary services were examined in this study. Table ,2 shows the veterinary services distribution of cattle. 1226 of cattle had veterinary services and 60 of cattle had no veterinary services. The prevalence among the veterinary services showed that a prevalence of 25.4 %, and those with no veterinary services had prevalence of 45,8% (Table, 3). The Chi- square test showed significant association between infection and availabiity veterinary services of animal (p- value = 0.024) (Table, 4).

3.1.7. Prevalence of bovine brucellosis among 1286 cattle examined by status of vaccination:-

A total of 1286 cattle of various vaccination were examined in this study. Table ,2 shows the vaccination of cattle. 965of cattle were vaccinated and 321 of cattle were not vaccinated . The prevalence among the vaccinated cattle showed that a prevalence of 28.3 %, and there were not vaccinated had prevalence of 18.4% (Table, 3). The Chi- square test showed significant association between infection and vaccination (p- value = 0.000) (Table, 4).

3.1.8. Prevalence of bovine brucellosis among 1286 cattle examined against history of abortion:-

A total of 1286 cattle of various history of abortion were examined in this study. (Table ,2) shows the history of abortion of cattle.289 of cattle had history of abortion and 997 of cattle had no history of abortion . The prevalence among the history of abortion showed that a prevalence of 28.4

%, and those had no history of abortion showed the prevalence of 25.1% (Table, 3). The Chi- square test showed no significant association between infection and history of abortion of animal (p- value = 0.259) (Table, 4).

3.1.9.Prevalence of bovine brucellosis among 1286 cattle examined against owner awareness:-

A total of 1286 cattle of various owner awareness were examined in this study. (Table ,2) shows the owner awareness of cattle. The prevalence among the owner awareness showed that had a prevalence of 28.3 %, and those were not awared had prevalence of 22.2% (Table, 3). The Chi- square test showed significant association between infection and owner awareness (p- value = 0.015) (Table, 4).

3.1.10. Prevalence of bovine brucellosis among 1286 cattle examined against mixed age:-

A total of 1286 cattle of various mixed age were examined in this study. (Table ,2) shows the mixed age of cattle. The prevalence among the mixed age showed that a prevalence of 13. %, and those were not had aprevalence of 26.% (Table, 3). The Chi- square test showed significant association between infection and mixed age (p- value = 0.158) (Table, 4).

3.1.11. Prevalence of bovine brucellosis among 1286 cattle examined by breeding method:-

A total of 1286 cattle of various breeding method were examined in this study.(Table ,2). The prevalence among the breeding method showed that artificial had a prevalence of 26. %, and natural had prevalence of 25.8.% (Table, 3). The Chi- square test showed no significant association between infection and breeding method (p- value = 0.972) . (Table, 4).

3.1.12. Prevalence of bovine brucellosis among 1286 cattle examined by bull sharing for breeding:-

A total of 1286 cattle of various bull sharing for breeding were examined in this study.(Table ,2) ,The prevalence among the bull sharing showed that a prevalence of 15.6. %, and those were not a prevalence of 26.6% (Table, 3). The Chi- square test showed significant association between infection and bull sharing (p- value = 0.021) (Table, 4).

3.1.13. Prevalence of bovine brucellosis among 1286 cattle examined by water source :-

A total of 1286 cattle of various water source were examined in this study. Table ,2 shows the water source of cattle. 301of cattle used tap water for drinking and 952 of cattle used water from well and 33 of cattle from common canal. (Table, 3)Show the rate of infection with the water source, 93(30.9%) cases were the use tap water, compared with 236(24.8%) cases used water source from well and 33(9.1) cases were used water from common canal. The Chi- square test showed significant association between rate of infection and water source (p- value = 0.009), (Table, 4).

3.1.14. Prevalence of bovine brucellosis among 1286 cattle examined by Housing:-

A total of 1286 cattle of various housing were examined in this study. Table (2) shows the housing of cattle.1066 of cattle were in intensive housing and 220 were in semi-intensive. (Table, 3)show the rate of infection with the housing, 288 (27%) cases were reported from intensive, compared with 44 (20%) cases from semi- intensive . The Chi-

square test showed significant association between rate of infection and housing (p- value = 0.030), (Table, 4).

3.1.15 Prevalence of bovine brucellosis among 1286 cattle examined by Age:-A total of 1286 cattle of various age were examined in this study. Table (2) shows the age of cattle .201 of cattle less than 3 year and 1085 of cattle were more than 3 year. (Table, 3) show the rate of infection with the age 13 (6.5%) cases were reported with age less than 3 years, compared with 319 (29.4%) cases from the age of more than 3years . The Chi- square test showed significant association between rate of infection and age (p- value = 0.000), (Table, 4).

3.1.16. Prevalence of bovine brucellosis among 1286 cattle examined by sex :-A total of 1286 cattle of various sex were examined in this study. Table (2) shows the sex of cattle .18 of cattle were male and 1268 of cattle were female. (Table, 3) show the rate of infection with the sex one case (5.6%) c reported when the sex was male, compared with 331 (26.1%) cases when the sex was female. The Chi- square test showed significant association between rate of infection and age (p- value = 0.048), (Table, 4).

Table 2: Summary frequency table for the distribution of 1286 serum samples examined by the RBPT according to potential risk factors

Risk Factor	Frequency	Relative frequency %	Cumulative frequency %
Localities			
Shergelneel	275	21.4	21.4
Bahary	312	24.3	45,7
Khartoum	144	11.2	56.9
Omdurman	238	18.5	75.4
Umbada	208	16.2	91.6
Karrry	109	8.5	
Total	1286		100.0
Herdtybe			
Cattle only	1039	80,8	
Mixed species	247	19,2	80.8
Total	1286	100.0	100.0
Breed			
Local	41	32	32
Cross	1245	96.8	
Total	1286	100.0	100.0
Veterinary Services			
Yes	1262	98.1	98.1
No	24	1.9	
Total	1286	100.0	100.0
Vaccination			
Yes	965	75.0	75.0
No	231	25.0	
Total	1286	100.0	100.0
Abortion History			
Yes	289	22.5	22.5
No	997	77.5	
Total	1286	100.0	100.0
Owner awareness			
Yes	760	59.1	59.1
No	526	40.9	
Total	1286	100.0	100.0

Table 2 continued

Breeding method			
Artificial	104	8.1	8.1
Natural	1182	91.9	
Total	1286	100.0	100.0
Bull sharing for Breeding			
No	1190	92.5	92.5
Yes	96	7.5	100.0
Total	1286	100.0	
Water Source			
Tap water	301	23.4	23.4
Well	952	74.0	97.4
Common canal	33	100.0	100.0
Total	1286		
Housing			
Intensive	1066	82.9	82.9
Semi-intensive	220	17.1	
Total	1286	100.0	100.0
Age			
Less than 3 years	201	15.6	15.6
More than 3 years	1085	84.4	
Total	12186	100.0	100.0
Sex			
Male	18	1.4	1.4
Female	1268	98.6	
Total	1286	100.0	100.0
Herd Size			
Less than 30	99	15.5	
30-60	316	24.5	15.5
More than 60	771	60.0	40.0
Total	1286	100.0	100.0

Table 3: Summary cross-tabulation for the prevalence of brucellosis with potential risk factors

Risk Factor	No. tested	No. positive	Percent
Locality			
Khartoum	144	48	33.,3
Omdurman	238	55	23.1
Karry	109	10	09.2
Umbada	208	64	30.8
Bahry	312	92	29.5
Shargelneel	275	63	22.9
Total	1286		
Herd tybe			
Cattle only	1039	290	27.90
Mixed species	247	42	17.00
Total	1286		
Breed			
Local	41	3	7.300
Cross	1245	329	26.40
Total	1286		
Veterinary services			
Yes	1262	321	25.40
No	24	11	45.80
Total	1286		
Vaccination			
Yes	965	273	28.30
No	321	59	18.40
Total	1286		
Abortion history			
No	997	250	25.10
Yes	289	82	28.40
Total	1286		
Owner awareness			
Yes	760	215	28.30
No	526	117	22.20
Total	1286		

Table 3 continued

Risk Factor	No. tested	No. positive	Percent
Breeding Method			
Artificial	104	27	26.00
Natural	1182	305	25.80
Total	1286		
Bull sharing for Breeding			
No	1290	317	
Yes	96	15	26.60
Total	1286		15.60
Housing			
Intensive	1066	288	27.00
Semi-intensive	220	44	20.00
Total	1286		
Age			
Less than 3years	201	13	6.50
More than 3years	1085	319	29.40
Total	1286		
water source			
Tap Water	301	93	30.90
Well	952	236	24.80
Common canal	33	33	9.10
Sex			
Male	18	1	5.60
Female	1268	331	26.10
Total	1286		
Herd size			
Less than 30	199	52	26.10
30-60	316	78	24.70
More than 60	771	202	26.20
Total	1286		

Table 4: Summary of univariate analysis for potential risk factors of bovine brucellosis in 1286 cattle examined in Khartoum state using the Chi- square test (χ^2)

Risk factors	No. tested	No. positive (%)	df	χ^2	P-value
1-Sex			1		
Male	18	1 (5.6%)		3.913	0.048*
Female	1268	331 (26.1%)			
2-Age			1		
<3 years	201	13 (6.5%)		48.57	0.00*
>3 years	1085	319 (29.4%)			
3-Breed			1		
Local	41	3 (7.3%)		7.568	0.06*
Cross	1245	329 (26.4(%)			
4- Herd size			2		
< 30	199	52 (26.1%)		0.281	0.869
30 -60	316	78 (24.7%)			
> 60	771	202 (26.2%)			
5- Herd type					
Cattle only	1039	290 (27.9%)	1	12.397	0.00*
Mixed species	247	42 (17 %)			
6-Vetrenary services			1	5.117	0.024*
Yes	1262	321 (25.4%)			
No	24	11 (45.8%)			
7-Vaccination			1	12.352	0.00*
Yes	965	273 (28.3%)			
No	321	59 (18.4%)			
8-Abortion istory			1	1.273	.259
Yes	289	82 (28.4%)			
No	997	250 (25.1%)			

Table 4 continued

Risk factor	No. tested	No. positive (%)	df	χ^2	P-value
8-Abortion History			1	1.273	0.259
Yes	289	82 (28.4%)			
No	997	250 (25.1%)			
9-Owner awareness			1	5.933	0.015*
Yes	760	215 (28.3%)			
No	526	117 (22.2%)			
10-Breeding Method			1	0.001	0.972
Artificial	104	27 (26.0%)			
Natural	1182	305 (25.8%)			
11-Bull sharing			1	5.626	0.021*
Yes	69	15 (15.6%)			
No	1190	317 (26.6%)			
12-water source			2	9.401	0.009*
Tap Water	301	93 (30.9%)			
Well	952	236 (24.8%)			
Common canal	33	33 (9.1%)			
13-Housing			1	4.688	0.030*
Intensive	1066	288 (27.0%)			
Semi-intensive	220	44 (20,0%)			
14-Locality			5	26.995	0.000*
Khartoum	144	48 (33.3 %)			
Omdurman	238	55 (23.1 %)			
Karary	109	10 (9.2 %)			
Umbada	208	64 (30.8 %)			
Bahry	312	92 (29.5 %)			
Shergelneel	275	63 (22,9 %)			

Table 5: Summary of Multivariate analysis for potential risk factors of bovine brucellosis in 1286 cattle examined in Khartoum state using Logistic Regression

Risk factor	No. tested	No. positive (%)	Exp(B)	95% C.I for Exp(B)	P-value
Locality					
Khartoum	144	48 (33.3)			0.00
Omdurman	238	55 (23.1)	1.223	0.644 -2.323	0.290
Karry	109	10 (9.2)	0.499	0.205 - 1217	0.000
Umbada	208	64 (30.8)	1.273	0.750 -2.159	0.075
Bahry	312	92 (29.5)	1.007	0.602 -1.683	0.089
Shergelneel	275	63 (22.9)	0 .790	0.452 - 1.382	0.014
Herd tybe					
Cattle only	1039	290 (27.9)			
Mixed species	247	42 (17.0)	0.611	0.388 - 0.981	0.000
Breed					
Local	41	3 (7.3)			
Cross	1245	329 (26.4)	3.731	1.058 -13.159	0.006
Veterinary Services					
Yes	1262	321 (25.4)			
No	24	11 (45.8)	2.148	0.880 –5.240	0.024
Vaccination					
Yes	965	273 (28.3)			
No	321	59 (18.4)	0.613	0.384 – 0.977	0.000
Owner awareness					
Yes	760	215 (28.3)			0.015
No	526	117 (22.2)	0.672	0.502 - 0.899	

Table 5 continued

Risk factor	No. tested	No. positive (%)	Exp(B)	95% C.I for Exp(B)	P-value
Bull sharing					
No	1290	317 (26.6)			0.018
Yes	96	15 (15.6)	0.911	0.466 – 1.780	
Housing					
Intensive	1066	288 (27.0)			0.030
Semi-intesive	220	44 (20.0)	0.749	0.471 – 1.191	
Sex					
Male	18	1 (5.6)			
Female	1268	331 (26.1)	7.643	0.995 -58.703	0.048
water source					
Tap Water	301	93 (30.9)			0.009
Well	952	236 (24.8)	0.592	0.414 - 0.844	0.056
Common canal	33	33 (9.1)	0.217	0.056 - 0.833	0.026
Age					
Less than 3years	201	13 (6.5)			
More than 3years	1085	319 (29.4)	6.230	3.469 -11.188	0.000

3.2.1. Estimation of the Financial Loss due to Bovine Brucellosis in Khartoum Locality:-

3.2.2.1. Estimation of the financial loss due to bovine brucellosis in the selected sample:-

1. Losses due to reduction of milk production:-

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

=14.6384 x 3.5= 51.2344 SDG.

2. Losses due to loss of aborted foeti:-

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

4 x4.500 + 4 x 35.00 = 32000 SDG

3. Loss due to repeat breeding :-

repeat breeding rate x seropositive animal = 0,08 x 48 = 3.84 cows=3 cows

Total loss in sample studied = 86.9544 SDG.

3.2.1.2 Estimation of the financial loss due to brucellosis in the herds studied.

Total loss in the herd studied= 86.9544 / 144 x716= 432.356 6 SDG.

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

Total loss in the whole locality = 86.9544/144 x 4114 = 2484.2389 SDG.

4.6 (SDG) per US dollar in (2015) from the Sudan bank. .(Anoon 2015).

Table (6). The total economic loss due to brucellosis in Khartoum L0clity

	SDG	U$$
Loss in milk	51.2344	
Loss in calve	32000	
Loss in fertility	3720	
Total Loss in the sample	86.9544	
Total Loss in herd Sample	432.3566	
Total in the Locality	**2.484238**	**6210591**

3.2.2. Estimation of the Financial Loss due to Bovine Brucellosis in Omdurman Locality:-

3.2.2.1. Estimation of the financial loss due to bovine brucellosis in the selected sample:-

1. Losses due to reduction of milk production:-

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

=17.5138 x 3.5= 59.54692 SDG.

2. Losses due to loss of aborted foeti:-

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

6 x4.500 + 6 x 35.00 = 48000 SD

3. Loss due to repeat breeding :-

repeat breeding rate x seropositive animal = 0,08 x 55 = 4.4 cows=4 cows

The financial losses due to repeat breeding = Number of repeat breeding cows x Cost of repeat breeding due to brucellosis/cow……..(3). 4 x 1.24 = 4.96 SDG.

Total loss in sample studied = 112,41692 SDG.

3.2.2.2 Estimation of the financial loss due to brucellosis in the herds studied.

Total loss in the herd studied= 112.41692 / 238 x1012= 478.00808 SDG.**3.2.2.3 Estimation of the financial loss due to brucellosis in the whole locality.**

Total loss in the whole locality =112.4169 2/23 8 x12072=52936.08848 SDG 4.6 (SDG) per US dollar in (2015) from the Sudan bank. .(Anoon 2015)

Table (7). The total economic loss due to brucellosis in Omdurman Loclity

	SDG	**U$$**
Loss in milk	59.54692	
Loss in calve	48.000	
Loss in fertility	4.96	
Total Loss in the sample	112,41692	
Total Loss in herd Sample	478.00808	
Total in the Locality	**5.2936.08**	**1.150782**

3.2.3. Estimation of the Financial Loss due to Bovine Brucellosis in Karry Locality:-

3.2.3.1. Estimation of the financial loss due to bovine brucellosis in the selected sample:-

1. Losses due to reduction of milk production:-

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

= 2.18655872 x 3.5 =7.65295552 SDG.

2. Losses due to loss of aborted foeti:-

Value of lost foet i= Number of aborted female foeti x price of female calf at weaning weight + Number of aborted male foeti x price of male calf at weaning weight

1 x4.500 + 1 x 35.00 = 8000 SDG

3. Loss due to repeat breeding :-

repeat breeding rate x seropositive animal = 0,08 x 10 =0 .8 cows= 0 cows

Cost of repeat breeding cows due to brucellosis = SDG 1.24/Cow (adapted from Angara and Elfadil,2014.Osman ,2015).

Total loss in sample studied = 15. 652955 SDG.

3.2.3.2 Estimation of the financial loss due to brucellosis in the herds studied.

Total loss in the herd studied = 15.652955 / 109 x487= 69.935679 SDG.

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

Total loss in the whole locality = 15.652955/109x9600= 1378.688 SDG

4.6 (SDG) per US dollar in (2015) from the Sudan bank. .(Anoon 2015)

Table (8). The total economic loss due to brucellosis in Karry Locality

	SDG	U$$
Loss in milk	7. 652955	
Loss in calve	8.000	
Loss in fertility	0.000	
Total Loss in the sample	15.652955	
Total Loss in herd Sample	69.935697	
Total in the Locality	**1378.688**	**344666**

3.2.4. Estimation of the Financial Loss due to Bovine Brucellosis in Umbada Locality:-

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

1. Losses due to reduction of milk production:-

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

= 20.9116 x 3.5 =73. 1906 SDG.

2. Losses due to loss of aborted foeti:-

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

8 x4.500 + 8 x 35.00 = 64.000 SDG

3. Loss due to repeat breeding :-

repeat breeding rate x seropositive animal = 0.08 x 64 = 5.12 cows= 5 cows

Cost of repeat breeding cows due to brucellosis = SDG 1.24/Cow (adapted from Angara and Elfadil,2014.Osman ,2015).

The financial losses due to repeat breeding = Number of repeat breeding cows x Cost of repeat breeding due to brucellosis/cow……..(3). 5 x 1.24 = 6.200SDG.

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

73. 1906 + 64.000 + 6.200 = 143.3906 SDG.

Total loss in sample studied = 143.3906 SDG.

3.2.4.2 Estimation of the financial loss due to brucellosis in the herds studied.

Total loss in the herd studied = 143.3906/ 208 x161 6= 1114.0346615 SDG.

4.6 (SDG) per US dollar in (2015) from the Sudan bank. .(Anoon 2015)

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

Total loss in the whole locality =143.3906/ 208 x20000=13.787.557 SDG .

Table (9). The total economic loss due to brucellosis in Umbada Locality

	SDG	U$$
Loss in milk	73.1906	
Loss in calve	64.000	
Loss in fertility	6.2000	
Total Loss in the sample	1433906	
Total Loss in herd Sample	11403466	
Total in the Locality	**13.787.557**	**2997950**

3.2.5. Estimation of the Financial Loss due to Bovine Brucellosis in Bahry Locality:-

3.2.5.1. Estimation of the financial loss due to bovine brucellosis in the selected sample:-

1. Losses due to reduction of milk production:-

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

=26.1441572x 3.5 = 91.504550 SDG.

2. Losses due to loss of aborted foeti:-

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

4 x4.500 +4 x 35.00 = 32000 SDG

3. Loss due to repeat breeding :-

Total loss in sample studied = 132.1845502 SDG.

3.2.5.2 Estimation of the financial loss due to brucellosis in the herds studied.

Total loss in the herd studied = 132.1845502 / 312 x3731 = 1580 706 SDG.

4.6 (SDG) per US dollar in (2015) from the Sudan bank. .(Anoon 2015)

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

Total loss in the whole locality = 132.1845502 / 312x3361 2= 14240.343SDG

Table (10). The total economic loss due to brucellosis in Bahry Locality

	SDG	U$$
Loss in milk	9150455	
Loss in calve	32.000	
Loss in fertility	8.6800	
Total Loss in the sample	132.18455	
Total Loss in herd Sample	1580 7069	
Total in the Locality	**14.240.343**	**3095726**

3.2.6. Estimation of the Financial Loss due to Bovine Brucellosis in Shergelneel Locality:-

3.2.6.1. Estimation of the financial loss due to bovine brucellosis in the selected sample:-

1. Losses due to reduction of milk production:-

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

= 17.5138 x 3.5 =61.2983 SDG.

2. Losses due to loss of aborted foeti:-

Value of lost foet i= Number of aborted female foeti x price of female calf at weaning weight + Number of aborted male foet x price

of male calf at weaning

weight……(2).

2 x4.500 + 2 x 3.500 = 1 6.000 SDG

3. Loss due to repeat breeding :-

repeat breeding rate x seropositive animal = 0.08 x 63 = 5.04 cows= 5 cows

repeat breeding = Number of repeat breeding cows x Cost of repeat breeding due to brucellosis/cow……..(3). 5 x 1.24 = 6.200SDG.

Total loss in sample studied = 83.49830 SDG.

3.2.6.2 Estimation of the financial loss due to brucellosis in the herds studied. Total loss in the herd studied = 83.4983/ 275 x2293= 696.22400690 SDG.

3.2.6.3 Estimation of the financial loss due to brucellosis in the whole locality. Total loss in the whole locality = 83.4983/ 275 x35460= 10766.726 SDG. 4.6 (SDG) per US dollar in from the Sudan bank. .(Anoon 2015).

Table (11). The total economic loss due to brucellosis in Shergelneel Locality.

	SDG	U$$
Loss in milk	61.29830	
Loss in calve	1 6.000	
Loss in fertility	6.2000	
Total Loss in the sample	8349830	
Total Loss in herd Sample	69.2240	
Total in the Locality	**10.766.726**	**2.340592**

3.3. Estimation of the financial loss due to brucellosis in the whole State

According to the results the highest economic losses in Bahry locality was 14240.343 SDG, Umbada locality was 13.787.557 SDG ,Shergalneel locality was 10.766.726 SDG ,Omrurman locality was 5.293.608 SDG , Khartoum locality was 2.484.239 SDG and Karry locality was 1.378.688 SDG

The mass losses was due to milk loss this is due to brucella effect on milk production , the highest economic losses of milk losses in Bahary locality 9.150455 SDG, Umbada l ocality was 7.319060 SDG, Shergalneel locality was 6.129830 SDG, Omdurman locality was 5.954692 SDG , Khartoum locality was 5.123442 and Karry locality was 765295 SDG.

In this study the economic losses of calves due to abortion was calculated in the State localities Khartoum, Omdurman, Karry, Umbada , Bahry, and Shergalneel 32,000 , 48.000 ,08,000 , 64.000 , 32,000, 16.000 SDG respectively

From the result the economic loss due to infertility due to brucella (repeat breeding) minor losses in localites Khartoum, Omdurman, Karry, Umbada , Bahry, and Shergalneel 3.726, 4.960 ,0,000, 6.200, 8.680, 6.200 SDG respectively.

Total economic loss in the whole State = Total economic loss in the whole localities=

2484.238 + 52936.0884 8 + 1378.68888 + 13787.55769 + 14240.3432 7+ 10766.72472

= 95593.643 SDG = **20781.226 U $$**

Table (12). The total economic loss due to brucellosis in Khartoum State..

Locality	Milk loss	Calve loss	Fertility loss	Herd loss	Total losses of Locality Loss
khartoum	51.23440	32.000	3.720	432.536	2.484.238
omdurman	59.54692	48.000	4.960	478.0080	5.293.608
Karry	07.65295	08.000	0.000	069.9356	1.378.688
Umbada	73.19060	64.000	6.200	1114.035	13.787.557
Bahry	91.50455	32.000	8.680	1580.706	14.240.343
sherglneel	61.29830	16.000	6.200	696.2240	10.766.726
Total	344.42772	200000	25760	3870.0808	9 5.593.643 SG 20781.226 U$$

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Chapter Four

Discussion

In the present study a brucellosis prevalence were observed in Khartoum State localities , the overall individual prevalence was 25.8% . RBPT and cELISA tests showed degree of agreement; however the variation in prevalence by the two tests could be due to false positive. RBPT has been described as a highly sensitive but not specific test, while the cELISA is both a specific and sensitivity test and can eliminate cross-reaction due to heterogeneous bacteria and can minimize false positive Nielsen *et al* (2007) . RBPT is less specific than the cELISA and the reported high prevalence(s) at herd level might be due to false-positive serum reactions (FPSR) and due to bias in farm selection. False-positive serum reactions in *Brucella* spp. screening tests are known to be caused by unrelated Enterobacteriaceae and cELISA can eliminate such reactions (Portanti , *et al* , 2006). *B. abortus* vaccination strain 19 also gives rise to an antibody response similar to that resulting from natural infection but cELISA can eliminate this false-positive reaction only by approximately 50% (Nielsen *et al* ,2007) .

This study showed a highest seroprevalence of brucellosis in cattle in localities of Khartoum state, Sudan as 35.4%; 31,2% ; 29.5%; 22.7%; 18.9% and 9.2% in Khartoum locality; Umbada ; Bahri ; Shargelneel ; Omduruman and Karri respectively. The overall seroprevalence result 25.8% is in agreement with the result of another study carried out in Khartoum State, Sudan, where the rate of infection in cattle was 25.7%

(Ebrahim ,2013) ; 25.3% (Ali, 2013) and 24.9% (Angra *et al* ,2009) . Higher than that(19.7%) individual prevalence reported by Abdalla *et al* (2012) in dairy farms around Khartoum State , and Mahmoud *et al* (2012) reported (8.4%) prevalence of the brucellosis in cattle in Eldein area, Eastern Darfur State, western Sudan. The present results are higher than that reported by Berhe *et al*, (2007) in the Extensive Cattle Production System of Tigray Region of Ethiopia, where he obtained(42.3%) herd prevalence. Also are higher than that reported by Ahamad *et al* (2009) in Jordan individual prevalence (6.5%) and herd prevalence (23%.) Kaoud *et al* (2010) in Egypt with(2.16%) individual prevalence and herd prevalence was (17.22%). Emanuel *et al* (2010) in Tanzania the overall seroprevalence was(5.75%) and herd prevalence was (15.9%). Also higher than that reported by Matope ,*et al* (2011) in Zimbabwe individual prevalence was (21.8%) and herd prevalence was (58.0%) . The present results of Khartoum State seroprevalence are higher than that reported by Muhammad *et al* (2011) in Islamabad Capital of Pakistan , with (20%) individual prevalence . Also higher than that reported by Anke *et al* (2013) in Melaka state of Malaysia with (2.5%) individual and (21.8%) herd prevalence .The present prevalence rate in Khartoum State (25.8%) is much higher than that reported by Nitu *et al* (2013) in India with (13.%) individual prevalence and in Tajikistan reported by Elisabeth .*et al* (2014) with 2.0% individual, (4.1%) herd prevalence .

The prevalence rate reported in this study is lower than that reported by Aggad *et al*,(2006) in Tiaret Province in western Algeria 26.3%.; in Uganda (25.9%) obtained by Makita *et al* (2011); in Ethiopia individual prevalence (26.1% ,) and 100% herd prevalence were observed by Bekele *et al* (2011); in Nigeria individual prevalence

(29.2%) were reported by Lawala *et al* (2012) and 26.3% were obtained by Mai *et al* (2012) , 28.13% in Egypt reported by Affi *et al* (2011).

In addition to investigating the prevalence, this study was conducted with the aim of identifying potential risk factors associated with brucellosis seroprevalence in cattle in Khartoum State, Sudan.

In this study, Herd size, Abortion History , Breeding Method were identified as the risk factors associated with seropositivity to *Brucella* antigens at the univariable analysis using chi-square test. No one of these three risk factors was significant in the multivariate analysis using logistic regression, suggesting the endemic status of brucellosis in and around Khartoum state may be maintained indefinitely by low-level within herd transmission.

This study disagree with many observations, Jergefa *et al*, (2009) reported a higher seroprevalence on farms that used artificial insemination opposed to those used natural mating. Chate *et al*, (2009) suggested that the risk factor associated with the presence of the infection was the use of artificial insemination, the risk of spread from the bull is much higher if the semen is used for artificial insemination. The difference in the herd sizes did not significantly affect the number of seropositive animals in this study. However, this is contrary to reports by some authors, who asserted that large herd size is one of the major risk factors for bovine brucellosis (Berhe *et al*. 2007). It has also been reported that a large herd size increases the exposure potential when a large number of animals are in contact with each other at common feeding and watering points, with higher risk following cases of abortion (Dinka & Chala 2009). None the less, the overriding factor for infection in this study may be common exposure of these animals,

irrespective of the herd size at watering and feeding points, particularly during the calving period.

The sex was found statistically significantly associated with *brucella* infection in cattle ($\chi^2 = 3.913$, $p < 0.048$). Where prevalence rate was higher (26.1%) and lower (5.6%) prevalence recorded in female and males cattle respectively . Similar to the findings in this study, other studies also recorded a higher seroprevalence in female animals than in male animals (Bekele *et al*. 2000; Berhe *et al*. 2007; Dinka & Chala 2009; Kebede 2008). According to Kebede *et al*. (2008), male animals are generally kept in the breeding herd for a shorter time than female animals, thus making the chances of exposure lower for male animals. Berhe *et al*. (2007) also stated that the serological response of male animals to *Brucella* infection is limited and that the tests of serologically positive male animals were usually observed to be culture negative.

The owner awareness was found statistically significantly associated with *brucella* infection in cattle ($\chi^2 = 5.93$ $p < 0.015$). Where prevalence rate was higher (28.3%) when owner have knowledge and lower (22.2%) prevalence recorded when owner have no knowledge. This knowledge was not a real knowledge because cattle attendants and professionals working in the area know nothing about precautions of brucellosis . Most cases of brucellosis in human are occupational and occur in the farm attendants, veterinarians, and butchers (Radostits et al .(2007).

The herd type was found statistically significantly associated with *brucella* infection in cattle ($\chi^2 = 12.397$, $p < 0.00$). Where prevalence rate was higher in dairy cattle than in mixed species. This is likely to be explained by the fact that a farmer usually keeps dairy cattle for long period

and brucellosis is known as a chronic disease and most of the farms rear cattle for milk production and also the numbers of mixed farms were low in the study.

In this study the prevalence of brucellosis was higher among the farm with no veterinary services (χ^2 =5.117, p <0.24). Veterinary services play a minor role in preventing the introduction of infection, while its role in preventing the spread of the infection inside the farms or herds is major. Poor veterinary service has been identified as a risk factor for brucellosis in Argentina (Samartino et al 2002) and Mexico (Luna et al, 2002). It is well known that delivering adequate animal health services results in a low incidence of diseases, and especially those diseases that have an infectious nature. The use of disinfectants and the presence of adequate veterinary services were identified as the factors that protect against bovine brucellosis . Ebrahem (2013).

In this study the breed was found statistically significantly associated with *brucella* infection in cattle (χ^2 = 7.568, p < 0.06). Where prevalence rate was higher (26.4%) when breed was cross breed and lower (7.3%) when breed was local breed . local breed have higher immunity than foreign breed. , the difference in breed-specific prevalence is contrary to the findings of Cadmus *et al*. (2010), who showed that the breed of cattle was not significantly associated with the disease

There is a significant difference between farms that shared male for breeding and farms that did not share male (χ^2 =5.626, p < 0.021).The prevalence of brucellosis increased in farm that used shared male for breeding than that not shared male for breeding. Brucellosis is a disease that is transmitted through genital tract (venereal transmission) from

infected male to female during service, when an infected bull is used for service of different farms, it may transmit the disease to animals in these farms Kaoud *et al*., (2010) suggested using of exogenous fertilizing system (OR = 3.2) was considered as very important risk factor for the introduction and spread of *Brucella* infection among farm animals. Ebrahem (2013).

The present results suggests that cows older than 3 years of age are more likely to become seropostive to *Brucella* (χ^2 =48.57, p < 0.00). A similar observation was made by other researchers . Cadmus *et al*. (2010), who showed that the , age-specific prevalence was higher in animals older than three years (8.8%) than in younger animals (2.8%), which is consistent with several reports (Bekele *et al*. 2000; Berhe *et al*. 2007). Sexually mature and pregnant cattle have been found to be more susceptible to infection by *Brucella* than sexually immature animals (Walker 1999). Younger animals tend to be more resistant to infection and frequently clear infections, although re-infection could occur at a later time (Radostits 1995). The higher prevalence of brucellosis in older cattle could be attributed to consistent exposure of the cattle to the infectious agent.

In this study the Vaccination was found statistically significantly associated with *brucella* infection in cattle (χ^2 = 5.117, p < 024). Where prevalence rate was higher in vaccinated cattle (28.3%) than in non vaccinated (18.4%) in this study. This may be due to some farms which were sampled during vaccination period .*Br. abortus* vaccination strain 19 also gives rise to an antibody response similar to that resulting from natural infection. Kaoud *et al*., (2010)

The Housing was found statistically significantly associated with *brucella* infection in cattle (χ^2 = 4.688, p < 0.030). Where prevalence rate

was higher in intensive- housing (27.00%) , lower in semi intensive – housing (20.00%) in this study, intensive- housing for cross breed which was lower immunity so that the prevalence rate was higher than local breed which there houses where semi intensive- housing and there immunity was high..

The water sources were found statistically significantly associated with *brucella* infection in cattle ($\chi^2 = 9.401$, $p < 0.009$). Where prevalence rate was higher in tap water (30.9%) and (24.8%) when source was well , lower in common canal (9.1%).This due to bad hygienic management with farm because most of them don't share a common source of water .Within farm all animal of different age , sex and multi spices share the same container.

In this study Khartoum state was proved to be endemic with brucellosis The highest economic losses most significant economic losses are usually incurred following bovine brucellosis. According to the result The highest economic losses in Bahry locality was 14.240.343 SDG, Umbada locality was 13.787.557 SDG ,Shergalneel locality was 10.766.726 SDG ,Omrurman locality was 5.293.608 SDG , Khartoum locality was 2.484.239 SDG and Karry locality was 1.378.688 SDG .

The mass losses was due to milk loss this is due to brucella effect on milk production , the highest economic losses of milk losses in Bahry locality 9150455 SDG that due to large number of dairy farm in Bahry locality with high productive animal and high percent of foreign breed which was highly sensitive to brucellosis ,Umbada locality was 7319060 SDG, Shergalneel locality was 6129830 SDG, Omdurman locality was 5954692 SDG ,Khartoum locality was 5123442 and Karry locality was 765295 SDG that due dairy population in Karry locality was few .

In this study the percentage of abortion was calculated in the state localities Khartoum, Omdurman, Karry, Umbada , Bahary, and Shergalneel ,0,04 , 0.06 ,0,01 , 0.08 , 0.04, 0.02 respectively .this indicate that there no new infection which causing storm of abortion. The remaining 0.08 suffered from infertility problem which actually not differentiated so is estimated depended on study by Angara and Elfadil (2014). From this result the loss due to infertility due to brucella (repeat breeding) constituted a minor percentage of total loss these indicate that the infertility due to brucellosis not a main factor of infertility in Khartoum State, farmers suffered from infertility problems generally due to other reasons, instead some of them keep the infertile cows without treatment and most of them get rid of the infertile cows by selling them (Osman,2015).
In Khartoum State Angara,(2005) estimated the cost of brucellosis in Kuku dairy scheme were the total losses accounted to SDG 66.910,503 equivalent to 267,642 U$$. Osman (2015) estimated In Jabel Aolia locality total economic losses due to brucellosis which accounted to 328,617,666 SDG equivalent to 71,439 U$$.The total losses in this study accounted to 9 5593.643,475 , SDG equivalent to 20781.226 U$$.

In Central America brucellosis has been estimated as between 4 and 8%, with higher prevalence in dairy herds and with losses calculated at US$ 25 million per year (Moreno, 2002). In Ethiopia, information on losses specifically through brucellosis in the different types of production systems is sparse, with the exception of Tariku (1994) who reported an annual loss from brucellosis estimated to be 88,941.96 Ethiopian Birr($5231equivalent) among 193 cattle,They was largely due to reduced milk production and abortions. The estimated total economic losses due to brucellosis stands at

about RM200,607,946.80 (USD 62,926,060.84) in a year for the whole of Malaysia (Pwaveno et al ,2012) . In Turkey total financial losses caused by brucellosis, respectively in optimistic, expected and pessimistic scenarios, were calculated as $20.066.875, $41.337.446 and $61.711.571(Can, et al ,2011).

Conclusions:

The results of this study demonstrated that bovine brucellosis is widely prevalent in the Khartoum State particularly in Khartoum; Umbada and Bahry localities . Herd size, Abortion History , Breeding Method were risk factors not significant in the multivariate analysis using logistic regression. Attributed the endemic status of brucellosis in Khartoum State. Our results could make a useful contribution towards preventing brucellosis in cattle and decreasing losses in the livestock industry. The study proved that brucellosis causes financial losses in dairy and meet production sector .

Recommendation:-

- The development of veterinary extension services in the state , is essential to promote awareness about brucellosis, its impact on livestock production and zoonotic risks, would provide a valuable prevention measure. This would help to unify both community/dairy cattle producers to control and eliminate brucellosis. Currently, many dairy cattle producers hide or dispose of animals with a history of abortion, potentially facilitating disease transmission between farms and regions. This seriously undermines efforts of controlling and preventing the disease
- An effort should be focused on educating animals owner for the importance of buying animals from herds free or herds officially free of brucellosis.

-- More research is needed on effective control of this infection in collaboration with farm associations, veterinarians and animals owner in order to establish an efficient control programme

- 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) . Test and culling of positive cows will reduce the prevalence of this disease especially in cattle.

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