بسم الله الرحمن الرحيم

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Prevalence and Risk Factors of Cattle Brucellosis in Atbara Locality-River Nile State

نسبه الإصابة وعوامل الخطر لمرض البروسيلا في الابقار في محلية عطبرة – ولاية نهر النيل

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BY

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قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ رَبِّي

لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ

جِئْنَا بِمِثْلِهِ مَدَدًا

الكهف - الآية 109-

Dedication

To my father, to my mother, brothers, sisters and to my only darling kid: Abdu Al maze.

With love and gratitude . .

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Firstly, my thanks are due to Almighty ALLAH for blessing and guidance for this work to be concluded. I would like to express my deep thanks to my supervisor: Dr. Isam Mahmoud for his keen supervision, direction and continuous interest and constructive criticism in reviewing the dissertation

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Abstract

Across sectional study was conducted to determine prevalence of Brucellosis in cattle and to investigate some associated potential risk factors from may2019 to October 2019 in Atbara locality, Nahr El Neel State. A total of 212 cattle serum samples were collected from farms El amn gzaei (113 animals), Elsyala (22 animals), Gzeira Nawawi (52animals) ,El fadlab (10 animals) and Um Eltour (15animals). They were examined by using Rose Bengal test for the presence of the anti bodis of bacteria . The results revealed that the prevalence rate of brucellosis in cattle1 was 3.8%.The questionnaire was used to determine the risk factors(sex ,age ,breed, hygiene ,size of hard and purpose of animal). The following risk factors showed association with cattle brucellosis in the univariate analysis under significant level of p-value ≤ 0.05: sex (p-value = 0.823), age (p-value =0.009), breed (p-value= 0.276), hygiene (p-value = 0.000), size of hard (p-value =0.000) and purpose of animal (p-value = 0.823). Using multivariate analysis to determine possible significant association between *Brucella* infection and potential risk factors, the result showed that there was significant association with age , hygiene and size of hard. It can be concluded that this *Brucella* infection was prevalent in a low percentage in Atbara locality. As a brucellosis is a zoontic disease its essential practices to pastoralizing milk and good cooking of meat before used is required for prevention in human.

ملخص البحث

أجريت دراسة مقطعية لتحديد معدل انتشار مرض اليروسيلا في الابقار ودراسة بعض عوامل الخطر المحتملة المرتبطة بها من مايو 2019 إلي اكتوبر 2019 في محلية عطبرة في ولاية نهر النيل . تم جمع 212 عينات سيرم من الابقار، من مزارع الامن الغذائي (113حيوان) ، السيالة (22 حيوان) الجزيرة نواوي (52 حيوان) الفاضلاب (10 حيوان) ام الطيور(15حيوان) ثم استخدام اختبار الروز بنقال لفحص الاجسام المضادة للبكتريا. وأظهرت النتائج أن عدوى البروسيلا كانت منتشرة بين الابقار في محلية عطبرة بنسبة انتشار 3.8%. تم استخدام الاستبيان لتحديد عوامل الخطر (الجنس، العمر، السلالة، الهيجين، حجم القطيع والغرض من الحيوان). وأظهرت عوامل الخطر التالية الارتباط مع مرض البروسيلا في تحليل وحيد المتغير تحت مستوى كبير من قيمة المعنوية ≤ 0.25 وكانت القيمة المعنوية لكل من الجنس (0.823)، العمر (القيمة الاحتمالية = 0.009), السلالة (القيمة الاحتمالية = 276.0)، hygiene (القيمة الاحتمالية = 0.000), حجم القطيع (القيمة الاحتمالية = 0.000)، الهدف من الحيوان (القيمة الاحتمالية = 0.823). باستخدام التحليل متعدد المتغيرات لتحديد احتمال وجود ارتباط كبير بين مرض البروسيلا وعوامل الخطر المحتملة وأظهرت النتيجة أن هناك ارتباط كبير مع العمرhygiene ، حجم القطيع. يمكن أن نستنتج أن عدوى البروسيلا كانت منتشرة في محلية عطبرة بنسبة بسيطة. ولأن المرض مشترك بين الحيوان والإنسان لابد من بسترة اللبن وطبخ اللحم جيداً قبل الاستخدام مطلوب لوقاية الانسان .

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Introduction

Brucellosis is a bacterial disease caused by species of the bacterial genus *Brucella* , named after Sir David Bruce who in1886 isolated the causative agent from soldier in Malta where the disease caused considerable morbidity and mortality among British military personnel (Morgan and MacKinnon, 1979; Halling and Young, 1994). In19th century, brucellosis was known as Malta or Mediterranean fever (Charters, 1980). These are non-motile, facultative and intracellular coccobacilli bacteria. They act as facultative intracellular bacteria (Barroso *et al*.,2002). There is six species of *Brucella* are important in animal *B. melitensis* reported in sheep and goats, *B. abortus* in cattle, *B. suis* in pigs, reindeer and small rodents, *B. canis is* found in dogs, *B. ovis* in sheep, and *B. neotomae* in desert wood rats. Recently, *B. pinnipedialis* (in seals) and *B. ceti* are newly reported species, infecting marine animals (Foster *et al*.,2007).

Brucellosis is a wide spread livestock infection in the middle East and north Africa(Foster *et al*.,2017).

Brucellosis affects domestic and wild animals and humans (Charters, 1980). B. abortus was first reported as a causative agent of cattle and intermittent fever in humans (Cutler *etal*., 2005 and Christopher *etal*., 2010). Brucellosis is an important human disease in many part of the world especially in the Mediterranean countries of Europe, North and East Africa ,Middle East ,south and central Asia and South America(Rahman *et al*.,2006). It is a zoonotic disease and the infection is almost invariably transmitted to people by direct or indirect contact with infected animals or their products. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs(WHO/OIE/FAO, 2006).

Brucella abortus is the principal cause of brucellosis in cattle (Radostits *et al.*, 2000; Abubakar and Arshed, 2012). Brucellosis is essentially adisease of

sexually mature animals. It mainly affects reproduction and fertility, reduces the survival rate of newborns and milk yield. There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be due to brucellosis (Munir *et al.,* 2010; Maadi *et al*., 2011).

Objectives of the study:

1. To determine the prevalence of brucellosis in Atbara locality.
2. To investigate some risk factors that increasing *Brucella*.
3. To obtain additional data on brucellosis in Atbara locality.

Chapter One

Literature Review

1.1. Scientific classification of Brucella:

Domain	Bacteria
Phylum	proteobacteria
Class	Alphaproteobacteria
Order	Rhizobiales
Family	Brucellaceae
Genus	*Brucella*
Species	*B.abortus* , *Br. Melitensis*, *Br.suis*

1.2. Brucellosis:

These bacteria are facultative intracellular, gram-negative, non-capsulated, non-flagellated and non-spore forming coccobacilli (Madigan and Martinko, 2006). The species *Br. Melitensis* contain three biovars *,Br.suis* four biovars and *Br. abortus* nine biovars. (Gwida *et al*., 2010). The predilection site of *Br.abortus* is the reproductive organs including placenta, aborted fetus and products of parturition, and it is most likely found in the milk, semen, feces and hygroma fluids (Glynn and Lynn, 2008).

1.2.1. Bovine brucellosis:

Brucellosis in cattle is caused almost exclusively by *B. abortus*. There are some areas where the coexistence of cattle and small ruminants facilitate cattle infection with *B. melitensis* (Samaha *et al*., 2008). Cattle can also become transiently infected by *B. suis* biovar 1 which prefer mammary glands (Olsen and Hennager, 2010).

1.2.2. Morphology:

Brucellosis is cause by organisms of the genus *brucella* the organism appear as coccbacilli or short rods measuring 0.5-0.7 um in width and 0.6-1um in length (Silver *etal.*, 2000).The morphology of *Brucella* is fairly constant except in old

cultures, where pleomorphic forms may be evident. *Brucella* are gram- negative and usually does not show bipolar staining. They are not truly acid-fast but resist decolouration by weak acid ,thus stained by the stamps modification of Ziehl- Nlesen method, which is some time used for the microscopic diagnosis of brucellosis from smear of solid or liquid specimens (Cloeckaert *et al*., 2001). In stained smear the organism appear single, in pair ,chains or spores and capsules (Weynant *et al*.,1997).

The *Brucella* have no classic virulence genes encoding capsules, plasmids, pili or exotoxins and compared to other bacterial pathogen relatively little is known about the factors contributing to the persistence in the host and multiplication within phagocytic cells. Also, many aspects of interaction between *Brucella* and its host remain unclear (Seleem *et al.,* 2008; Sriranganathan *et al.,* 2010).

1.2.3. Epidemiology:

The epidemiology of cattle brucellosis is influenced by several factors including factors associated with disease transmission between herds, factors influencing the maintenance and spread of infection within herds (Crawford *et al*. 1990).

1.2.4. Geographic Distribution:

B. abortus was once found worldwide in cattle, with rare exceptions such as Iceland. Eradication programs in a number of European nations, Canada, Australia, New Zealand, Japan and Israel have eliminated this organism from domesticated animals. The U.S. is also *B. abortus*-free, with the exception of one region described below. Sporadic cases may be reported in travelers and immigrants in *B. abortus*-free countries.

Wildlife reservoirs for *B. abortus* are known to exist in parts of Africa and North America. In north America, this organism is maintained in bison and elk in the Greater Yellowstone Area in the U.S., and bison in the Canadian Wood

Buffalo National Park and an adjacent area of the Northwest Territories in Canada. In the U.S., a possible additional reservoir has been identified in feral pigs in South Carolina. Infected bison in Canada are separated from cattle by a buffer zone, however, wildlife occasionally transmit *B. abortus* to livestock in the U.S., where cattle are grazed on open ranches and public lands near infected wildlife hosts. There is no evidence that any wild ungulates are infected in European countries that have eradicated *B. abortus(*OIE2018*)*.

1.2.5. Host Factor:

Under natural infection may occur in animals of all age groups ,but persists commonly in sexually mature animals. Generally, infection is acquired after three Years of age with increase in the sub sequent age groups (Rajesh *etal*.,2003).some study results revealed the equal distribution of *Brucella* antibodies among males and females .In other finding it appeared that females are more susceptible to the disease than male(Silver *etal*.,2000).

1.2.6. Distribution in Africa:

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange free state of South Africa in 1915 (Chukwu, 1985). However**,** still the epidemiology of the disease in livestock and humans as well as appropriate preventive measures are not well understood and such information is inadequate particularly in sub Saharan Africa. The importance of brucellosis reflects its widespread distribution and its impacts on multiple animal species, including cattle, sheep, goats, pigs and humans. While the importance of brucellosis is widely assumed, the benefits of programs to control it, relative to their costs, need to be assessed. (Mc Dermot *et al*., 2002).Some countries in Africa where seroprevalence of brucellosis had been reported to be less than 10% were Benin 4.3%, Ethiopia 4.2%, and Ghana 6.6% (Kubuafor *et al*., 2000; Megersa *et al*., 2011).

1.2.7. Brucellosis in Sudan:

Sudan is the largest Arab and Afrian country, it is surrounded by nine countries and divided in to 26 state one of them is River Nile state. It is possesse a great livestock population of cattle which estimated as (108538) (Anon, 2018)

The River Nile state divided in to seven Localities one of them is Atbara Locality. The estimation of cattle live stock in it is about(6512) (Anon, 2018)

In Sudan ,cattle brucellosis was reported in all part of country and the prevalence rate was found to be higher compared to other animal species. The first incidence of bovine brucellosis was reported from a dairy herd in Khartoum where B. abortus was isolated from an aborted cow. In Eastern Sudan camel brucellosis was firsty reported by Mustafa and Nur in1968, the prevalence was ranged between 0.1and 5.5%(Omer, 2006).

Human brucellosis was diagnosed in the Sudan as early as1904 in a patient at Berber, (Haseeb,1950).

1.2.8. Transmission:

1.2.8.1. Transmission in animals:

Transmission occur between cattle, an infected animal must be excreting brucellae. Excretion is almost entirely limited to the time period immediately following abortion or full-term parturition with high numbers of *B. abortus* present in uterine, fluid and within the placenta. Three factors determine the likelihood of transmission to susceptible animals: the number of brucellae excreted during parturition, the survival of these bacteria in the environment, and the probability of a susceptible animal being exposed to enough brucellae to establish infection. It is estimated that 105CFU of *B. abortus* are sufficient to induce infection in 78% of naïve cattle via conjunctival exposure (Manthei, 1968). Slightly more bacteria are likely necessary to infect animals via an oral route of exposure. One gram of placental tissue from an infected cow is estimated to contain 20-360 infectious doses (Olsen and Johnson, 2011).

The level of shedding may vary depending on a number of factors. Shedding typically decreases with each subsequent parturition following infection. Persistence of *B. abortus* in the environment, and thus the duration of time during which naïve animals can be exposed to brucellae following a parturition event, is dependent on environmental conditions. Survival of *Brucella* is enhanced by the presence of moisture and lower temperatures.

Brucella abortus can survive an estimated 180 days within a fetus in the shade, 5-150 days in water, 8-240 days in manure, and 151-185 days in cold, moist soil. Survival in direct sunlight is only 4.5 hours (Crawford *et al.*, 1990).

The final factor determining transmission to susceptible animals, probability of exposure, depends largely on husbandry practices. If allowed the opportunity, cattle will often investigate, smell, or ingest placental material left on the pasture or barn floor. The oral route is considered the primary means by which susceptible animals are exposed, although in intensively farmed cattle housed indoors, the conjunctival route is also likely important.

Vertical transmission is also a common route of infection. An estimated 20% of surviving calves are infected *in utero*. *Brucella*-contaminated milk is another potential source of infection for calves (Crawford *et al.*, 1990 and Nicoletti,1980). *Brucella* infection in bulls can manifest as orchitis, epididymitis, and seminal vesiculitis, and localization of brucellae in these organs typically results in shedding in the semen. However, when used for natural mating, infected bulls are considered a negligible source of infection for naïve cows. *Brucella* contaminated semen is of considerable risk to naïve cows bred by artificial insemination (Crawford *et al.*, 1990). The difference in risk maybe due to the different locations in which semen are deposited. It is possible that brucellae are unable to survive within the cervix due to the presence of anti-microbial factors (Nicolette, 1980).

Although a minor source of exposure overall, dogs may play important roles in *B. abortus* epidemiology in some areas. Dogs are often kept on farms and if given the opportunity will consume bovine placental material. High levels of *B. abortus* infection have been documented in dogs in some locations for example in regions of Mongolia 36% of dogs are seropositive (Zolzaya *et al.*, 2014). *Brucella abortus* can cause abortions in pregnant dogs, and the resulting environmental contamination has been reported to cause infection of cattle kept in close proximity (Crawford *et al.*, 1990, Nicoletti, 1980).

1.2.2.8. Zoonotic transmission:

Zoonotic transmission occurs most frequently via unpasteurized milk products in urban settings, while occupational exposure of farmers, veterinarians, or laboratory workers can result from direct contact with infected animals or tissues or fluids associated with abortion (Olsen and Palmer, 2014). Only rare cases of vertical and horizontal (Wyatt, 2010) transmission between humans have been reported (Ruben *et al*., 1991; Mantur *et al*., 1996; Çelebi *et al*., 2007; Meltzer *et al*., 2010) and humans are generally considered to be incidental, or dead-endhosts for *Brucella* species (Meltzer *et al*., 2010). The spillover of brucellae from wildlife to domestic ruminants is also possible (Mick *et al*., 2014).

1.2.9. Pathogenesis:

The major route of infection is through mucous membranes of the oropharynx and upper respiratory tract or conjunctiva (Tabak *et al*., 2008). Another route is through the mucous membrane of the male and female genital tract. On entering into the body of the host, the organism encounters the cellular defenses of the host but generally succeed in arriving via the lymph vessels at the nearest lymph node after escaping the cellular defenses (Kho and Splitter, 2003). The fate of the invading bacteria is mainly determined by cellular defenses of the

host chiefly macrophage and T-lymphocytes though specific antibody also plays apart (Radostits *et al*., 2007).

In contrast to other pathogenic bacteria, *Brucella* lack classical virulence factors, such as exotoxins, cytolysins, capsules, fimbria, plasmids, lysogenic phages, drug resistant forms, antigenic variation, but possibility that they might have unique and subtle mechanisms to penetrate host cells, elude host defenses, alter intracellular trafficking to avoid degradation and killing in lysosomes and modulate the intracellular environment to allow long-term intracellular survival and replication (Moreno and Moriyon, 2002; Delrue *et al*., 2004).

Brucella uses a number of mechanisms for avoiding or suppressing bactericidal responses inside macrophages. The smooth lipopolysaccharides that cover the bacterium and proteins involved in signaling, gene regulation, and trans-membrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al*., 2005).

When the bacteria prevail over the host‟s defenses, a bacteremia is generally established. The bacteremia is always detected after 10 to 20 days and persists from 30 days to more than two months. If the animal is pregnant, bacteraemia often leads to the invasion of the uterus (Olsen& Tatum, 2010). At the same time, infection becomes established in various lymph nodes and organs, often in the udder and sometimes in the spleen (WHO, 2006) .

1.2.10. Clinical Signs:

1.2.10.1. Incubation Period:

The period between infection and reproductive losses is variable, as animals can be infected at any time (including before they become pregnant), but abortions usually occur late in gestation.(OIE, 2018)

1.2.10.2. Clinical Signs in animals:

Abortions (typically during the second half of gestation), stillbirths and the birth of weak offspring are the predominant clinical signs in cattle. Weak calves

may die soon after birth. Most animals abort only once, and subsequent pregnancies are usually normal. Lactation may be decreased. Clinical signs of mastitis are generally absent although *B. abortus* is shed in the milk. Uncomplicated reproductive losses are not usually accompanied by signs of illness; however, retention of the placenta and secondary metritis are possible complications. Epididymitis, seminal vesiculitis, orchitis or testicular abscesses are sometimes seen in bulls. Infertility or reduced fertility occurs occasionally in both sexes, due to metritis or orchitis/ epididymitis. Arthritis and hygromas may also be seen, especially in long-term infections. Deaths are rare except in the fetus or newborn. Infections in nonpregnant cows are usually asymptomatic (OIE, 2018).

Infection acquired by calves at birth may be temporary or develop into latent infection. Heifer calves that develop latent disease remain asymptomatic and serologically negative until first parturition at which time abortion and seroconversion are frequently observed (Wilesmith, 1978, Nicoletti, 1980).

1.2.10.3. Clinical description in human:

Incubation Period :

Highly variable (5 days–6 months) and Average onset betwen 2–4 weeks .

Symptoms **:**

Acute non-specific: Fever, chills, sweats, headache, myalgia, arthralgia, anorexia, fatigue, weight loss .

Sub-clinical infections are common

Lymphadenopathy (10–20%), splenomegaly (20–30%)

Chronic stage **:**

Recurrent fever .

Arthritis and spondylitis .

Possible focal organ involvement (as indicated in the case definition) (CDC).

1.2.10.4. Post Mortem Lesions:

Aborted fetuses may appear normal, be auto lyzed, or have evidence of a generalized bacterial infection, such as excess serohemorrhagic fluid in the body cavities and subcutaneous tissues, bronchopneumonia, fibrinous pleuritis, and an enlarged spleen, liver and lymph nodes. The placenta may be edematous and hyperemic, and exudate may be present on its surface. The placentomes can be variably affected, with some having no gross lesions, and others with severe necrosis and hemorrhage. The intercotyledonary areas are often thickened.

Epididymitis, orchitis and seminal vesiculitis, with inflammatory lesions, abscesses or calcified foci, may be observed in males. The tunica vaginalis may be thickened, with fibrosis and adhesions. In chronic cases, the testes can be atrophied. Some females may have metritis, with lesions that can include nodules, abscesses, fibrinous necrotic exudates and hemorrhages. Abscesses and granulomatous inflammation can sometimes be found in other organs and tissues, especially the lymph nodes, liver, spleen, mammary gland, joints, tendon sheaths and bones. Hygromas may be detected in some animals (OIE, 2018).

1.2.11. Diagnosis:

1.2.11.1. Bacteriological method of diagnosis:

1.2.11.1.1. Stained smears:

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) method. The presence of large aggregates of intracellular, coccobacillus red organisms is presumptive evidence of brucellosis. It is still often used, even though this technique is not specific as other abortive agents such as *Chlamydophila abortus* or *Coxiella burnetii*are also stained red (Alton *et al*., 1988; FAO, 2006).

1.2.11.1.2. Cultural isolation:

Definitive diagnosis of brucellosis is based on culture, serologic techniques or both. Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view (Bricker, 2002; Al Dahouk, 2003). However, in spite of its high specificity, culture of *Brucella* spp. is challenging. *Brucella* spp. is a fastidious bacterium and requires rich media for primary cultures. Furthermore, its isolation requires a large number of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory (Seleem *et al*., 2010;Hadush *etal*., 2013).

In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as fetal gastric contents, milk, vaginal secretions and semen are samples of choice for isolation. Milk samples should be a pool from all four mammary glands. Non- pasteurized dairy products can also be sampled for isolation (Lage *et al*., 2008; Poester *et al*., 2010). Reliable samples for isolation purposes in necropsied animals include mammary, iliac, pharyngeal, parotids and cervical lymph nodes, and spleen. Samples must be immediately sent to the laboratory, preferentially frozen at -20°C, and they must be identified as suspect of *Brucella* spp. infection (Poester *et al*., 2010).

Contamination of samples is complicating factor for *Brucella* spp. isolation. Therefore, the use of nutrient-rich media supplemented with antibiotics is used to inhibit growth of contaminants that may prevent isolation of *Brucella* spp. (De Miguel *et al*., 2011).Another limiting factor for culturing *Brucella* spp. is ther equirement for appropriate laboratory conditions and personnel training so the procedure can be performed safely (Nielsen and Ewalt, 2004). *Brucella* spp. is classified as a Biosafety level 3 organism, whose manipulation should be performed in biosafety level-3 laboratories (Lage *et al*., 2008).

For the isolation of *Brucella* spp., the most commonly used medium is the Farrell medium (FM), which contains antibiotics able to inhibit the growth of other bacteria present in clinical samples. However, due mainly to the nalidixic acid and bacitracin contained in its formulation, FM is inhibitory for *B. ovis* and also for some *B. melitensis* and *B. abortus* strains (Marin *et al*., 1996).

A new selective medium (CITA) containing vancomycin, colistin, nystatin, nitrofurantoin, and amphotericin B was found to be more sensitive than FM (De Miguel *et al*., 2011). The cream and the sediment part of the milk obtained after centrifuge are spread on to the surface of at least three plates of solid selective medium. Placenta and other solid tissues need to ground manually or homogenize in a blender or stomacher with a small proportion of sterile water. Fetal stomach contents are collected, after opening the abdomen, bysearing the surface of the stomach with a hot spatula and aspirating the liquid contents with a Pasteur pipette or syringe. As some *Brucella* species, like *B. abortus* biovars 1-4, need CO2 for growth the culture plates should be incubated at 35°C to 37°C in 5% to 10% CO2. *Brucella* colony may be visible after 2-3days, but cultures are usually considered negative after 2-3 weeks of incubation (Alton *et al*., 1988).

1.2.11.1.3. Biotyping:

Biotyping of *Brucella* spp. is performed using different tests, like agglutination tests with antibodies against rough (R antigen) or smooth LPS (against the A or M antigens); lysis by phages, dependence onCO2 for growth; production of H2S; growth in the presence of basal fuchsine or thionine; and the crystalviolet or acriflavine tests (Alton *et al*., 1988).These techniques must be carried out using standardized procedures by experienced personnel and usually performed only in reference laboratories.

1.2.11.2. Serological diagnosis:

Serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infection1. The tests are

crucial for laboratory diagnosis of brucellosis since most of control and eradication programs rely on these methods. Despite the development of numerous serological tests, no single test identifies all infected animals and a wide variation exists in estimates of their diagnostic accuracy (Abernethy *et al*., 2012; Adone and Pasquali, 2013). The serological tests are presumptive diagnosis for brucellosis in animals as well as human (OIE, 2012).

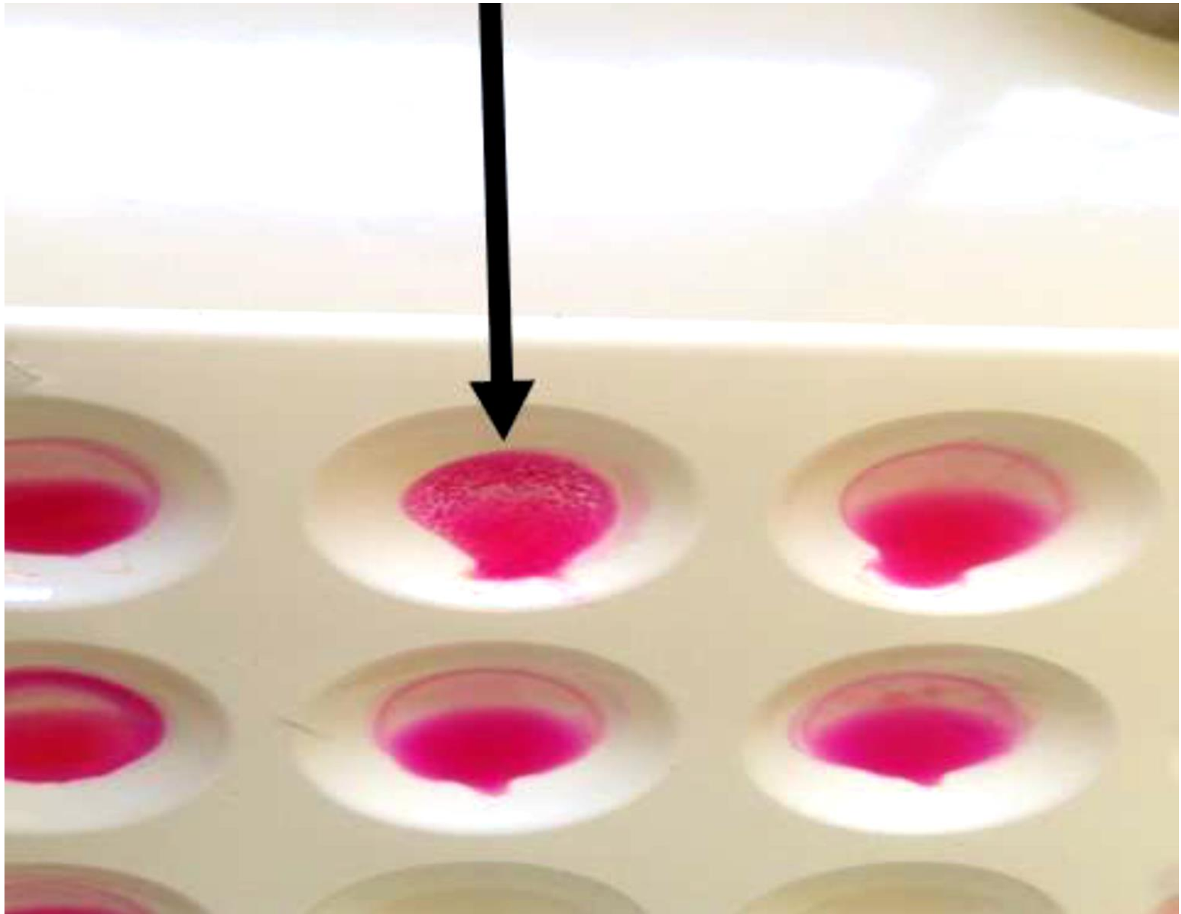
Serology can help to diagnose clinical cases or screen herds. Serological tests can determine that an animal has antibodies to a *Brucella* species with "smooth" LPS in the cell wall, such as *B. abortus*, *B. melitensis* or *B. suis*; however, they cannot distinguish reactivity to different organisms within this group. Commonly used tests in cattle include the buffered *Brucella* antigen tests (rose Bengal test and buffered plate agglutination test), complement fixation, indirect or competitive ELISAs and the fluorescence polarization assay. (OIE,2018)

1.2.11.2.1. Rose Bengal plate test:

This test was developed by Rose and Roekpe (1957) for the diagnosis of bovine brucellosis to differentiate specific *Brucella* agglutinins from non-specific factors. It is a rapid, slide-type agglutination assay performed with a stained *B. abortus* suspension at pH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources. It does not differentiate between field and S19 vaccine strain reactions and low sensitivity particularly in chronic cases (Díaz *et al*., 2011). The overall sensitivity is 92.9%, so the use of RBT should be considered carefully in endemic areas, particularly in individuals exposed to brucellosis and those having history of *Brucella* infection (Ruiz-Mesa *et al*., 2005).

As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution (1:2 through 1:64) of the serum samples (Christopher *et al*., 2010). World Health Organization (WHO)

guidelines recommend to confirmationing the RBT by other assays (Araj, 2010., Christopher *et al*., 2010).



Picture (1.1) Rose pingal test

1.2.11.2.2. Complement fixation test:

Due to its high accuracy, complement fixation is used as confirmatory test for *B. abortus*, *B. melitensis*, and *B. ovis* infection s and it is the reference test recommended by the OIE for international transit of animals (Gall *et al*., 2001; OIE, 2009). In most cases, the CFT is used on RBPT positive sera, but like the RBPT. The test has disadvantages such as high cost, complexity for execution, and requirement for special equipment and trained laboratory personnel. Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100% (Gall *et al*., 2001; Perrett *et al*., 2010). The reagents include *B.*

abortus CFT antigen, complement, amboceptor (haemolysin), ovine erythrocytes and test serum with Vernal buffer as the diluents (WHO, 2006; IBM, 2013).

1.2.11.2.3. Enzyme linked immunosorbent assay:

Enzyme linked immunosorbent assay (ELISA) has become popularas a standard assay for the diagnosis of brucellosis serologically . It measures IgG, IgA and IgM antibodies and this allows a better interpretation of the clinical situation. The diagnosis of brucellosis is based on the detection of antibodies against the smooth LPS. Detection of IgG antibodies is more sensitive than detection of IgM antibodies for diagnosing cases of brucellosis but specificity is comparable (Araj, 2010; Sathyanarayan *et al*.,2011; Agasthya *et al*., 2012). Comparing the conventional agglutination methods, ELISA is more sensitive in acute and chronic cases of brucellosis and it offers a significant diagnostic advantage in the diagnosis of brucellosis in endemic areas. This test is an excellent for screening large populations for *Brucella* antibodies and for differentiation between acute and chronic phases of the disease (Gall *et al*., 2003). It is the test of choice for complicated, local or chronic cases particularly when other tests are negative while the case is under high clinical suspicion. It can reveal total and individual specific immunoglobulins (IgG, IgA and IgM) within 4-6 hours with high sensitivity and specificity. In addition to the detection of immunoglobulin classes,ELISA can also detect *Brucella*-specific IgG subclasses and other *Brucella* immunoglobulin such as IgE (Araj, 2010).

The indirect ELISA has also been used for diagnosis using serum or milk from cattle (Gall *et al*., 2003; Di Febo *et al*., 2012). O ELISA-i has been usually used for smooth LPS *Brucella* spp., and it is sensitive and specific for *B. abortus* or *B. melitensis*, but it is not capable of differentiating antibodies induced by the vaccine strains S19 or Rev1(Lim *et al*.,2004; Ko ky *et al*., 2012). Sensitivity of i-ELISA varies from 96 to 100% and its specificity from 93.8% and 100% (Gall *et al*., 2001; Gall and Nielsen, 2004). On the other hand competitive enzyme

immunoassays were developed in order to eliminate some, but not all of the problems arising from residual vaccinal antibody, and from cross-reacting antibodies. The assays are carried out by selecting a monoclonal antibody with slightly higher affinity for the antigen than most of the vaccinal /cross-reacting antibody, but with lower affinity than antibody arising from infection (Munoz *et al.*, 2005; OIE, 2009; Poster *et al.*, 2010). The specificity of the competitive enzyme immunoassay is very high and is able to detect all antibody is otypes (IgM, IgG1, and IgG2 and IgA) (Nielsen, 2002). However, it is slightly less sensitive than the indirect enzyme immunoassay. This assay is an outstanding confirmatory assay for the diagnosis of brucellosis in most mammalian species.

1.2.11.2.4. Serum agglutination test (SAT):

The SAT has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques.

1.2.11.2.5. Supplementary tests:

Many other serological tests have been employed. Some, such as the Rivanol or 2-ME test, are variable from SAT and, although more specific, sharemany of its disadvantages. At present, the use of such procedures in the place of the standard test is not advised.

1.2.11.2.6. Milk testing:

In dairy herds, milk is an ideal medium to test as it is readily and cheaply obtained, tests can be repeated regularly and give a good reflection of serum antibody. Milk from churns or the bulk tank can be screened to detect the presence of infected animals within the herd which can then be identified by blood testing. This method of screening is extremely effective and is usually the method of choice in dairy herd.

1.2.11.2.6.1. Milk ring test:

The milk ring test (MRT) is a simple and effective method, but can only be used with cow's milk. A drop of haematoxylin-stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd.Non-specific reactions are common with this test, especially in brucellosis free areas. The milk ELISA is far more specific than the MRT.

1.2.11.2.6.2. Milk ELISA:

The ELISA may be used to test bulk milk and is extremely sensitive and specific, enabling the detection of single infected animals in large herds in most circumstances.

1.2.11.2.7. Fluorescence polarization assay:

This technique, which requires special reagents and reading equipment, is claimed to have advantages in sensitivity and specificity over other methods. Evaluation has been limited however, and the procedure is not widely available. Further information is required before its overall value can be assessed.

1.2.11.2.8. Intradermal test:

In this procedure, using a standardized antigen preparation such as Brucellin INRA or Brucellergene OCB, for monitoring the status of herds in brucellosis-free areas. It is sensitive and specific but false positive reactions can occur in vaccinated animals.(WHO, 2006)

1.2.11.3. Molecular methods for Brucella species genotyping:

Molecular techniques are important tools for diagnosis and epidemiologic studies, providing relevant information for identification of species and biotypes of *Brucella* spp., allowing differentiation between virulent and vaccine strains (Le Flèche *et al*., 2006; López-Goñi *et al*., 2008). Molecular detection of

Brucella spp. can be done directly on clinical samples with-out previous isolation of the organism. In addition, these techniques can be used to complement results obtained from phenotypic tests (Bricker, 2002). Despite the high degree of DNA homology within the genus *Brucella*, several molecular methods, including PCR, have been developed that allow, to a certain extent, differentiation between *Brucella* species and some of their biovars (OIE, 2009).

1.2.11.3.1. Polymerase chain reaction (PCR) :

Polymerase chain reaction (PCR) is the most broadly used molecular technique for brucellosis diagnosis (Bricker,2002). The technique is chosen based on the type of biological sample and the goal, i.e., diagnosis or molecular characterization or epidemiological survey. Most of the molecular diagnostic methods for brucellosis have sensitivity ranging from 50% to 100 and specificity between 60% and 98%. The DNA extraction protocol, type of clinical sample, and detection limits of each protocol, are factors that can influence the efficiency of the technique (Mitka *et al*.,2007).

Since the routine identification and differentiation of brucellosis suspected specimens, based on culture isolation and phenotypic characterization, requires Biosafety level-3 (BSL-3) protocols for the high risk of laboratory-acquired infections (Boschiroli *et al*., 2001).

1.2.11.3.2. Multiplex polymerase chain reaction typing :

Several multiplex PCRs which identify the genus *Brucella* at the species level and partly at the biovar level using different primer combinations have been reported. The first multiplex PCR, called AMOS PCR assay (AMOS is an acronym from „„*abortus-melitensisovis- suis*""), comprised five oligonucleotide primers for the identification of selected biovars of four species of *Brucella*. A new multiplex PCR assay (Bruce-ladder) has been proposed for rapid and simple one-step identification of *Brucella*. The major advantage of this assay over previously described PCRs is that it can identify and differentiate in a single step

most *Brucella* species as well as the vaccine strains *B. abortus* S19, *B. abortus* RB51 and *B. melitensis* Rev.1. In contrast to other PCRs, Bruce-ladder is able to detect also DNA from *B. neotomae*, *B. pinnipedialis* and *B ceti*. In addition, *B. abortus* biovars 3, 5, 6, 7, 9, and *B. suis* biovars 2, 3, 4, 5 can be identified by this new multiplex PCR. The only minor inconvenience of the Bruce-ladder is that some *B. canis* strains can be identified erroneously as *B. suis* (López *et al*., 2011).

1.2.11.3.3. Real-time PCR:

Real-time PCR is more rapid and more sensitive than conventional PCR. It does not require post amplification handling of PCR products, thereby reducing the risk of laboratory contamination and false positive results. Real-time PCR assays have been recently described inorder to test *Brucella* cells in urine, blood, and paraffin-embedded tissues (Redkar *et al*., 2001; Kattar *et al*., 2007).

1.2.12. Prevention and Control:

1.2.12.1 Management

Bovine brucellosis is usually introduced into a herd in an infected cow, but it can also enter in semen from infected bulls and on fomites. As the disease often goes undetected the identification of infected herds and animals is of primeimportance (Aulakh *et al*. 2008).

Herd replacements should be seronegative and should come from brucellosis-free regions or herds. If such herds are unavailable in an endemic are vaccinated calves or nonpregnant heifers are considered to be the safest option. Herd additions should be quarantined and re-tested before being released into the herd. Some infected animals, especially animals latently infected when they were young, might not be detected by either serology or culture. Semen for artificial insemination should only be collected from *Brucella*-negative animals that are tested regularly.(OIE, 2018).

In an infected herd, the placenta, any abortion products and contaminated bedding should be removed promptly and destroyed. Where feasible, areas exposed to infected animals and their discharges should be cleaned and disinfected. The offspring of infected animals should not be used as herd replacements due to the risk that they may be latently infected. *B. abortus* can be eradicated from a herd by test and removal procedures, or by depopulation. Programs to eradicate this organism from a country also include movement controls on infected herds, surveillance and tracing of infected animals (OIE, 2018).

1.2.12.2.Vaccination:

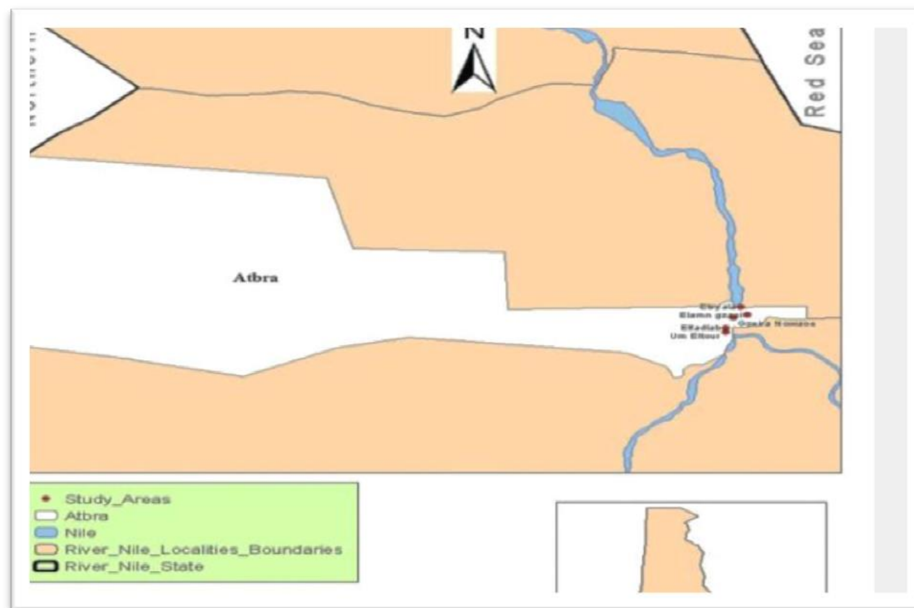
In different parts of the world both live vaccines, such as *B. abortus* S-19, *B. melitensis* Rev1, *B. suis* S-2, rough *B. melitensis* strain M111 and *B. abortus* strainRB51 and killed vaccines, such as *B. abortus* 45/20 and *B. melitensis* H.38 are available. Using of the RB51 attenuated live vaccine has recently gained popularity for control of brucellosisin cattle (Cheville,*et al*., 1996).

CHAPTER TWO

Materials and Methods

2.1. Study area:

River Nile State lies approximately between 22-35 longitude, east 16-22 latitude North ,and extend from Elsabuloga near River Nil south toward Bayoda desert to the Northern state North (Mohammed *et al*.,1996). Atbara is a town located in River Nile State in North Eastern Sudan (Atbara, 2007). It is located at the junction of the Nile and Atbara rivers .It is an important railway junction and rail road manufacturing center ,and most employment in Atbara is related to the rail lines .It is known as the Railway city ,and The National Railway Company's Headquarters are actually located in Atbara. The city is also a home to one of Sudan's largest cement factories(Atbara cement Corporation). The State surface area is about 124,000Km^{2}and the climate is that of a dry hot desert annual rainfall varies from zero in Northern part to150mm in the Southern .Trees and grasses such as *Acacia*, *Cherenbergiana and Aristida spp*. represent natural pastures beside the irrigation land around the river bank which is considerably small (Mohammed *et al* .,1996).



Map(2.1) River Nile State

2.2. Study design :

Across sectional study was conducted on El amn gzaei, Elsya, Gzeira Nowaoe,El fadlab and Um Eltour Animals were selected randomly to determine the prevalence of *Brucella spp* in cattle .

Prevalence of cattle *Brucella* was calculated by the following equation (Farooq *et al* ., 2012).

$$\text{Prevalence Rate} = \frac{\text{No of cattle with Brucella}}{\text{Total No of cattle tested at particular point in time}} \times 100$$

2.3. Sample size:

Regular visits were made by investigator in cattle farms . A total of 212 cattle blood samples were examined .The survey period extended from April 2019 to October 2019.During the collection of the samples the age ,sex, breed ,hygiene, size of herd and purpose of animal were recorded . The age of animals was determined for both sexes based on dentition, those animals with the age less than three years were considered as young while those greater than or equal to three were considered as old .

The sample size was calculated according to Thrusfield (2005) formula by using 50% expected prevalence with 5% absolute precision at 95% confidence interval.

$$n = \frac{Z^2 * Pexp(1- Pexp)}{d^2}$$

Where, n= the sample size.

Pexp = expect prevalence (0.5).

d = desired absolute precision (usually 5%) .

z = required confidence level, (Z=1.96 for 95% confidence interval) .

Therefore, by substituting the values of variables in the formula the sample size was determined to be 212, which is used as representative animal on which the study was done to know the prevalence of brucellosis.

2.4. Data collection :

Materials:

Ice box.

Bench centrifuge .

Centrifuge tube to separate the serum.

Different laboratory consumable.

Rose Bengal antigen and plates.

Refrigerator.

The data were collected through observation structured questionnaires that target the key persons in the farms of Atbara locality selected .Moreover the samples (212) were collected using probability random sampling techniques.

2.5. Questionnaire Survey:

A structured questionnaire with the primary objective of elucidating the multi factorial background of *Brucella* in cattle was conducted in an interactive manner at each farms.(six) structured questionnaire were filled out by asking the owners. The form including sex, breed, hygiene, size of hered, purpose of animal, and age .

2.6. Statistical analysis :

Frequency table of the distribution according to potential risk factor was constructed . Cross tabulation of brucellosis according to potential risk factor was made . Univarate analysis by the Chi –square test using statistical packets for Social Sciences (SPSS). Multivariate analysis by Logistic Regression models to perform risk factor significant at level ≤ 0.25 in the Unviarate model .The significant level in the Multivariate analysis was be ≤ 0.05.

Chapter Three

Result

The results indicated natural *Brucella* infection was prevalent among Sudanese cattle at Atbara locality (River Nile State) with an overall prevalence of 3.8 % .

Among 212 cattle serum samples examined 8 animals were found positive but 204 animals were found to be negative for cattle brucellosis (Table 3.1) .

Table 3.1: Prevalence of *Brucella* infection among 212 cattle examined in Atbara locality .

	Frequency	Percent	Valid Percent	Cumulative Percent
Negative	204	96.2	96.2	96.2
Positive	8	3.8	3.8	100.0
Total	212	100.0	100.0	

Table 3.2: Summary of frequency of examined cattle(n=212) for brucellosois in Atbara locality .

Risk factor	Frequency	Frequency(%) Relative	Cumulative Frequency (%)
Sex			
Male	5	2.4	2.4
Female	207	97.6	100.0
Breed			
Local	31	14.6	14.6
Cross	181	85.4	100.0
Hygiene			
Good	188	88.7	88.7
Poor	24	11.3	100.0
Age			
Old	92	43.4	43.4
Young	120	56.6	100.0
Size of herd			
Large	146	68.9	68.9
Small	66	31.1	100.0
Purpose of animals			
Beef cow	5	2.4	2.4
Milking cow	207	97.6	100.0

Sex of animals:

Among 207female cattle examined 8 were found positive for B*rucella spp* indicating prevalence of .3.9 % . Among 5 male animals examined 0%were found positive for *Brucella spp* indicating prevalence of.0 % (Table 3.3).

The Chi-square test showed that there was no significant association between *Brucella* infection (Table :3.4) and sex of animals (P-value0.823) .

Breed of animals:

The total number of cross animals examined were181,the positive were 8animals and the rate of infection was4.42%.The local cattle examined were 31animals and the number of infected animals were 0% with rate of infection 0.0% (Table 3.3).

The Chi-square test showed that there was no significant association between *Brucella* infection (Table :3.4) and breed of animals (P-value0.276) .

Hygiene of enviroment:

The animals with good hygiene were188 animals and 1(0.5%)were infected .While the animals with poor hygiene were24 animals and 7(29.2%) were infected (Table 3.3).

The Chi-square test showed that there was significant association between *Brucella* infection (Table :3.4) and hygiene
of animals (P-value0.000) .

Age of animals:

The results of age showed that the total number of young less than 3 years were 92 .The animals infected were 0 and the rate of infection was.0%.The total of the old animals were (above than 3 years) 120.The animals infected were 8the rate of infection was 6.7%. (Table 3.3).

The Chi-square test showed that there was significant association between *Brucella* infection (Table : 3.4) andAge of animals (P-value0.009).

Size of herd of animals:

The results of size of herd showed that the total number of small less than 10 were 66 , the animals infected were 8 and the rate of infection was12.1%.
The total number of large herd more than10 were146 the animal affected were 0 and the rate of infection was 0.0%.
The Chi-square test showed that there was highly significant association between *Brucella* infection (Table :3.4) and size of hared of animals(P-value0.000).

Purpose of animal:

The results of beef cow showed that the total number of 5 animals were tested .The animals infected were 0.0 and the rate of infection was0.0%.The total of the milking animals were 207.The animals infected were 8 the rate of infection was 3.9 %. (Table 3.3).

Table 3.3: Summary cross - tabulation of Brucellosis in 212examined cattle at Atbara locality.

Rate infected %	Animals affected	Animals tested	Risk factors
Sex			
Male	5	0	0.0%
Female	207	8	3.9
Breed			
Local	31	0	0.0%
Cross	181	8	4.24%
Hygiene			
Poor	24	7	29.2%
Good	188	1	0.5%
Age			
Old	120	8	6.7%
Young	92	0	0.0%
Size of herd			
Large	146	0	0.0%
Small	66	8	12.1%
Purpose of animals			
Beef cow	5	0	0.0%
Milking cow	207	8	3.9%

Table 3.4 Summary of analysis for potential risk factors of Brucellosis in Cattle examined in Atbara locality using Chi- square test.

Risk factor	No. inspected	NO. affected %	DF	X^2	P-value
Sex					
Male	0	5	1	0.201	0.823
Female	8	207			
Breed					
Local	0	31	1	1.424	0.276
Cross	8	181			
Hygiene					
Poor	7	24	1	48.059	0.000
Good	1	188			
Age					
Old	8	120	1	6.374	0.009
Young	0	92			
Size of herd					
Large	0	146	1	18.391	0.000
Small	8	66			
Purpose of animals					
Beef cow	2.4	5	1	0.201	0.823
Milking cow	97.6	207			

Means significant value .p-value ≤ 0.05.

Table 3.5: Multivariate analysis of Brucellosis and potential risk factors in 212 cattle examined at Atbara locality using logistic Regression.

Risk factor	Animals affected	Exp (B)	95% Confidence Interval for Exp (B)		P- Value
			Lower Bound	Upper Bound	
Age					
Old	120(6.7)	0	9.722	9.722	0.469
Young	92(0)	20.695	0	0	
Size of herd					
Large	146(0)	20.857	0	0	0.00
Small	66(12.1)	0	1.143	1.143	
Hygiene					
Poor	24(29.2)	0	0	0	0.093
Good	188(0.5)	1.946	0.722	67.84	

*Means significant value P-value ≤ 0.25.

CHAPTER FOUR

Discussion

Cross-sectional study, attempted to look the status of bovine brucellosis in Atbara locality in the River Nile state , Northern Sudan . The study reveals that, the animal level prevalence of bovine brucellosis was found to be 3.8%**.** This relatively low prevalence might be attributable to no extensive grazing conditions, these could reduce both animal to animal contact and the contamination of pastures under dry climatic conditions (Crawford *et al*., 1990; Adugna *et al*., 2013). This finding was lower compared with previous study in Bahr el Ghazal State, Sudan which was 6.5% (Nuol *etal* .,2018) .On the other hand ,it is mostly higher than the result reported in Bangladesh which was 2.4 (Rahman *et al*. , 2006).

The prevalence of *Bruella* in cattle according to sex was estimated in these study , the rate of infection in males was 0%and in females was3.9%.There was significant association between the sex and the disease (P-0.000), The study also revealed that all positive animals were females. This finding agreed with the reports by Kebede *et al*., (2008); Tolosa *et al*., (2008) and Dinka and Chala, (2009) also agreed with that reported in Indian Sharma *et al.,*(2003) and disagreed with Hailemelekot *etal*., (2007). Female animals are maintained in herds over extended time period thus, have ample time for exposure to the pathogen and being source of infection for other animals (Megersa *et al*., 2011 and Adugna *et al*., 2013). Other explanation for this finding could be that the number of male animals in each herd was low and were mostly reared separately, thus the chance of exposure is lower for males.

Regarding the breed distribution of the bacteria (*Brucella),* the current finding revealed that the prevalence in local and cross breed of animals was 0.0% + 4.42% respectively ,the highest infection was found in cross breed this could be due to, limited number of local breed animals in this study because of

their low number in extensive production system ,there was no statistically significant association(P-.276). This study in agreement with Jergefa *et al*. (2009) this is due to the compounded effect of management systems in cross-breed, and dis agree with Moti *et al*. (2012).

The present study also revealed that the prevalence of bovine brucellosis was significantly associated with the age of the cattle (P-0.009).The highest infection was found in more than three years (Older animals). Brucellosis appears to be more associated with sexual maturity (Radostits, 2007), and higher prevalence is repeatedly reported in sexually matured animals. This result is agrees with the report of Asfaw *et al*.(1998); Bekele *et al*.(2000); Omer *et al*. (2000); Jergefa *et al*.(2009) and Asmare *et al*.(2010).

There was highly statistically significant variation in the prevalence of Brucellosis and hygiene (P-0.000) in present study, an animal with poor hygiene seems to have higher prevalence(29.2%) of brucellosis than prevalence was in good hygiene(0.5%)may be due to abetter immunity in good hygiene.

This study also indicated that there was significant association of the prevalence of Brucellosis in different size of herd (P-0.000), The highest rate of infection was found in small(12.1%), in large the rate of infection was(0%).This in finding disgreement with the previous reports (Asfaw *et al*., 1998;Tolosa *et al*., 2008; Asmare *et al*., 2010; Adugna *et al*., 2013). This could be due to different in management in large herd the management was good and mating also was controlled.

The prevalence of *Brucella* in cow according to Purpose of animals was estimated in these study , the rate of infection in milking cow was %3.9 and in beef cow was0.0%.There was no significant association between the Purpose of animals and the disease (P-.823) and highest rate of infection was found in milking cow .

Conclusion and Recommendation

Conclusion:

The output of this study indicates , that the overall prevalence of brucellosis was 3.8% . The presence of high rate of *Brucella spp* in the area was responsible for the loss of production in cow .A high prevalence of infection was in females as compared to males .Old animals were highly effected as compared to young animals .A high prevalence of infection was in cross compared to local breed . A high prevalence of infection was in poor hygiene as compared to good hygiene . A high prevalence of infection in milking cow compared to beef cow. A high prevalence of infection was in small size of herd as compared to large size of herd.

Recommendation:

* The distribution of disease (brucellosis) in all Sudan states should be studied through a future plan .

* Improvement of husbandry practices in cattle farms .

* Community educational program should be carried out targeting brucellosis in the areas to aware livestock owners as well as general public in order to avoid direct or in direct contact with infected animals and their products.

* Control measures such as isolation or culling of aborted animal, proper disposal of aborted fetus, pasteurization or boiling of milk before consumption should be carried out to reduce risk of infection and transmission of the disease in livestock and human in the study area.

* Good personal hygiene is very important to prevent human infection .

Reference

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Appendix I

Frequency table for the distribution of infection among 212 cattle examined at Atbara locality according to potential risk factors.

A. Frequency distribution Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	5	2.4	2.4	2.4
	female	207	97.6	97.6	100.0
	Total	212	100.0	100.0	

B.Frequency distribution Breed

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	local	31	14.6	14.6	14.6
	cross	181	85.4	85.4	100.0
	Total	212	100.0	100.0	

C.Frequency distribution Hygiene

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	good	188	88.7	88.7	88.7
	poor	24	11.3	11.3	100.0
	Total	212	100.0	100.0	

D.Frequency distribution age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yong	92	43.4	43.4	43.4
	old	120	56.6	56.6	100.0
	Total	212	100.0	100.0	

E. Frequency distribution size of herd

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	large	146	68.9	68.9	68.9
	small	66	31.1	31.1	100.0
	Total	212	100.0	100.0	

F.Frequency distribution purpose of animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	beef cow	5	2.4	2.4	2.4
	mlking cow	207	97.6	97.6	100.0
	Total	212	100.0	100.0	

Appendix II

Cross-tabulation for the distribution of infection among 212cattle examined at Atbara locality according to potential risk factor.

A. Cattle Brucellosis and Sex Cross - tabulation

Count		Sex		Total
		male	female	
Result	negative	5	199	204
	positive	0	8	8
Total		5	207	212

B. Cattle Brucellosis and Breed Cross tabulation

Count		Breed		Total
		local	cross	
Result	negative	31	173	204
	positive	0	8	8
Total		31	181	212

C. Cattle Brucellosis and Hygiene Cross - tabulation

Count		Hygiene		Total
		good	poor	
Result	negative	187	17	204
	positive	1	7	8
Total		188	24	212

D. Cattle Brucellosis and Age Cross - tabulation

Count		age		Total
		young	old	
Result	negative	92	112	204
	positive	0	8	8
Total		92	120	212

E. Cattle Brucellosis and Size of herd Cross – tabulation

Count		size of hared		Total
		large	small	
Result	negative	146	58	204
	positive	0	8	8
Total		146	66	212

F. Cattle Brucellosis and Purpose of animal of hared Cross - tabulation

Count		purpose of animal		Total
		beef cow	milking cow	
Result	negative	5	199	204
	positive	0	8	8
Total		5	207	212

Appendix III

Univarate analysis for the association of cattle brucellosis in 212 cattle with potential risk factors using Chi-square test.

A. Sex:

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.201[a]	1	.654
Likelihood Ratio	.389	1	.533
Linear-by-Linear Association	.200	1	.655
N of Valid Cases	212		

B. Breed :

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.424[a]	1	.233
Likelihood Ratio	2.583	1	.108
Linear-by-Linear Association	1.417	1	.234
N of Valid Cases	212		

C. Hygiene

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	48.059[a]	1	.000
Likelihood Ratio	26.686	1	.000
Linear-by-Linear Association	47.832	1	.000
N of Valid Cases	212		

D. Age

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.374[a]	1	.012
Likelihood Ratio	9.345	1	.002
Linear-by-Linear Association	6.344	1	.012
N of Valid Cases	212		

E. size of herd

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	18.391[a]	1	.000
Likelihood Ratio	19.377	1	.000
Linear-by-Linear Association	18.304	1	.000
N of Valid Cases	212		

F. Purpose of animals

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.201[a]	1	.654
Likelihood Ratio	.389	1	.533
Linear-by-Linear Association	.200	1	.655
N of Valid Cases	212		

Appendix IV

Questionnaire for data collection to investigate the risk factors which could be associated with *Brucella* in Atbara locality .

*Animal No ( )

1. Breed of animal:

1- Cross ( ) 0- Local ( )

2. Age of animal :

0- less than two years (young) 1- More than Two years (old)

3.Sex of animal :

0- Male ( ) 1- Female ( )

4. Size of herd :

0- large ( ) 1- smal ( )

5- Purpose of animal :

0- Beef cow ( ) 1- Milking cow ( )

6- Hygiene:

0 Good ( ) 1 poor ( )

7- Result:

0- Negative ( ) 1- Positive ( )